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PROCEEDINGS OF SEMINAR ON THE SIGNIFICANCE OF FECAL COLIFORM IN INDUSTRIAL WASTES, MAY 4-5, 1972

Robert H. Bordner, et al

National Field Investigations Center
Denver, Colorado

July 1972

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ENVIRONMENTAL PROTECTION AGENCY

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SEMINAR ON
THE SIGNIFICANCE OF FECAL COLIFORM IN INDUSTRIAL WASTES

MAY 4-5, 1972

EDITED BY
ROBERT H. BORDNER & BOBBY J. CARROLL

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DENVER, COLORADO
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CLEAN WATER

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SEMINAR ON
THE SIGNIFICANCE OF FECAL COLIFORM IN INDUSTRIAL WASTES

MAY 4-5, 1972

Edited by

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EPA Seminar

The Significance of Fecal Coliform in Industrial Wastes

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Introduction and Summary

On May 4 and 5, 1972, at the invitation of the National Field Investigations Center-Denver, a group of EPA microbiologists, sanitary engineers, and other concerned participants held a seminar to discuss the significance of fecal coliforms in certain industrial wastes and the use of bacteriological parameters as effluent standards in the permit program. Additional topics that were discussed at the meeting included the relevance of *Klebsiella* as an indicator of sanitary quality, the need for standardization of methods within EPA, and improved communication among agency microbiologists.

The seminar included six formal presentations pertinent to the subject of the meeting. Open discussion followed each presentation and a general discussion period was held at the end of the seminar session. The participants made specific recommendations for monitoring nutritive industrial wastes and reached the following general conclusions:

1. The fecal coliform test is the most valid microbiological parameter for industrial wastes presently available.
2. The excessive *Klebsiella* densities in fecal-coliform test results from certain industrial wastes such as the pulp and paper, sugar beet, and food-processor effluents are significant because they reflect the high nutrient levels in these wastes. They can suppress *E. coli* detection, they can be pathogenic, and they are coliforms by definition. In addition, *Klebsiella pneumoniae* is found in the intestinal tract of approximately 30 percent of humans and 40 percent of animals.

3. Nutrient-rich wastewaters provide the capability for bacterial re-growth in the receiving streams.

4. *Salmonella* are recoverable from a number of high nutrient wastes. They are also capable of re-growth and of producing a potential public health hazard. However, pathogen detection alone is not adequate for monitoring because no one test will detect all possible pathogens that might be present, and negative results do not ensure the absence of all pathogenic organisms.

5. Effluent standards for highly nutritive, industrial wastes shall be below 30 mg/l and preferably in the range of 14-30 mg/l, as measured by BOD or the equivalent TOC. A TOC level was not recommended, pending the establishment of a value that will correlate with the recommended BOD limits.

6. The fecal-coliform bacteria test shall be used to monitor the nutrient reduction, by measuring the bacterial levels in the treated wastes. A geometic mean density of fecal coliforms, not to exceed 1,000/100 ml, was recommended as an index of adequate treatment. Bacteriological analyses will be performed on final effluents prior to disinfection, if disinfection is required.

7. When the receiving waters are classified for contact sports or shellfish harvesting, the bacteriological and chemical limits shall be reduced in order to comply with the established water quality standards.

8. The discharger has the primary responsibility for conducting the required monitoring. EPA or other agencies may need to provide analytical support for small industries. Preferably EPA will conduct the initial monitoring in order to provide basic data and to evaluate the performance of the proposed system. At a later date the industries or other agencies may assume the monitoring responsibility, with periodic checks by EPA.

9. Sampling should be conducted initially at a higher frequency, such as weekly or biweekly covering maximum flow periods. After adequate baseline data have been attained, less frequent samplings may be adequate.

10. High priority should be given to research devoted to the improvement of bacteriological and chemical parameters for industrial wastes. Improved tests for the indicator organisms and methods for pathogen isolation are needed. Further research should be conducted in order to define the public health risk associated with *Salmonella*, *Klebsiella*, and other pathogens in industrial waste discharges.

11. The importance of standardization of methods within the agency was emphasized.
12. Better communication among agency microbiologists was wholeheartedly endorsed. The seminar was in favor of periodic meetings to deal with microbiological problems.
13. Suggestions were made for needed additions and supplementation to Standard Methods.

Statement of Problem

Thomas P. Gallagher

The National Field Investigations Centers (NFIC) have been assigned the responsibility for preparing effluent guidelines that can be used by the Permit Program in developing permits under either the Refuse Act or, in the event of its passage, the new water pollution legislation. We have had meetings with technical representatives of some of the industries, namely the pulp and paper industry and the canning industry. Many of the effluent guidelines that we are preparing for the industries contain a fecal coliform or a coliform bacteriological constraint. The pulp and paper representatives as well as the sugar beet people have questioned the sanitary significance of fecal coliform and whether or not it can be required in developing a permit. To them it has some very practical significance in terms of the economics involved. Generally the industries have not been reqired to put in disinfection equipment and to hold down their coliform levels with the kinds of rather expensive installations that would be necessary if some of the standards in the effluent guidelines are accomplished.

About two months ago in Corvallis the pulp and paper people presented at a seminar what has been reported to me as a rather strong case against coliform standards for this industry. We felt that, because of the casethat had been made in Corvallis, it was necessary to assemble the best people in EPA who had familiarity or experience with this problem and *see whether there is sufficient evidence and sufficient* *technical rationale for requiring a coliform or some kind of bacteriological standard in the effluent guidelines that will be written into the permit program. Now let me tell you this: it must be a technically defensible standard and it must be backed up by evidence.* We've got to stay away from things that are "desirable" or that "we would like to see." It must have strong technical substantiation.

Now, on that basis, we have Murray Stein, who is the Chief Enforcement Officer for Water Control and in charge of the Enforcement Proceedings Division in Washington. He will have a lot to say about what happens with this problem.

(Mr. Stein is recognized.)

I want to welcome you here, and if you need anything, just let either Robert Schneider or William Stang know and they will accomodate you. We want to have a real free-wheeling discussion. We have a tape recorder, but it's not being used to inhibit anybody. As a matter of fact, we plan to assemble the results of these discussions in a report and send it to you, the active participants, for comment, and forward it to Headquarters for recommendation on this whole problem, at least from the technical people within EPA.

I'm going to get out of the way and Richard Bauer, from Region X, who has had some experience in this particular problem, will moderate this discussion. We'd like to hear presentations from the participants and then we would like to have a rigid question-answer session and discussion back-and-forth on the particular information, and a decision on whether or not you think what's been presented is defensible. Thank you.

Bauer: Thank you. It's a real pleasure to be here today. I would like to start by all of us introducing ourselves.

(Attendees proceed to introduce themselves. See preceding list of participants and attendees.)

I hope personally that this is the first of a number of meetings of this sort. I think that we can profit a great deal by, to use an old cliche, " that cross-fertilization of having people who are working in the field, discuss the kind of problems that they are facing," and, if this turns out to be a profitable experience, I hope that perhaps we can make this an annual affair.

In any event, we have a policy that was tentatively formulated by the NFIC, and I believe copies of it have been passed out. [See Appendix A: Considerations for Permit Preparation, Paper and Pulp Industry.] I feel that the most important purpose of this meeting is to try to come to some agreement as to whether this policy is relevant. If there are any difficulties with it, will you point them out to Mr. Stein? Again, I agree completely with Tom Gallagher that the policy can't be based upon personal feelings; we should be able to back up what we say with numbers and some hard data, because the industry is vitally concerned with this problem and is willing to go to the wall concerning it.

If we can dispose of this issue in the next two days, or day and a half, I think it would also be relevant while all of us are here, to enter into some discussions on the adequacy of Standard Methods. There have been some complaints made about it from various people. I think this would be a good opportunity to discuss that, and then perhaps a general discussion on ideas that people in the field have, to help us in our enforcement. I know a number of people are using new techniques in bacteriological analysis for enforcement purposes like fluorescent antibody techniques, enteropathogenic *E coli* identification and that type of thing.

With that in mind, I would like to kick the meeting off with a little historical background on the subject, "The Significance of Fecal Coliforms in Industrial Wastes."

History & Background on Occurrences of Fecal Coliforms in Industrial Wastes

R. Richard Bauer

I first became involved with the questioning of the relevance of coliform tests to pulp mills in about 1968 when I was assigned to NERC, Corvallis. The State of Oregon had adopted a rather ambitious program for waste treatment on the Willamette River. This included both municipal and industrial wastes. It required an implementation plan that all the industry and municipalities be on-line with secondary treatment and with adequate disinfection for the municipalities by 1972. The municipalities had to have their treatment by 1968. All the municipalities complied, and all the bacteriological standards were still fractured rather badly. We were experiencing total coliform counts, in numerous places throughout the river, on the order of 100,000/100 ml. Fecal coliform values were considerably lower, by and large, and on the order of magnitude of approximately 50.

Coincidentally, about this time the National Council for Air and

Stream Improvement (hereafter referred to as the National Council) had funded some research. A gentleman by the name of Ching Chang, in a master's thesis at John Hopkins, came to the conclusion that total coliform tests were not relevant. I think that most of us agree that certainly there is some question on its applicability. Mr. Chang recommended that we go to a fecal coliform standard.

Since then the National Council has also challenged the fecal coliform standard, and the basis for their challenge is a series of studies they did on these pulp and paper mills on the Willamette River. They selected mills in which, they claimed, they completely separated all their domestic sewage. They dumped dye down all the toilets, and checked all the plumbing, and I am convinced that they tried quite hard; in all probability, domestic sewage is being excluded from these plants.

Nevertheless, the coliform levels being discharged in the effluent are extremely high. In one particular case, Crown-Zellarbach's sulfite mill (Lebanon, Oregon), total coliforms were consistently 100,000,000/100 ml and fecal coliform values are on the order of 100,000. They went further to start IMViC-typing all these total and fecal coliforms. From the fecal coliform plates, in all cases except Crown-Zellarbach, they claimed they found absolutely no *E coli*. They will admit to having isolated some Type I *E coli* out of Crown-Zellarbach (Lebanon). They have done some additional testing to try to isolate *Salmonella* out of the wastes. They were unsuccessful, and came to the position at that time that the fecal coliform test is not relevant.

This was argued between the State of Oregon, the National Council, and the EPA Water Laboratory (Corvallis). It was decided at that time to form an ad hoc committee to see if we could shed some light on this problem. We started getting together in October 1969 to try to coordinate our research. Although not a researcher, I am capable of applying logic to some basic bacteriological questions and I, with the concurrence of the National Council, the states of Oregon, Washington, and Idaho (we included them in this at this time), and some of the food processors, put together a proposal to study five mills on the Willamette River to see whether we could isolate any potentially pathogenic organisms out of the wastes, to study the effect on the receiving water, and to ascertain if we could define the areas that need further research.

I now refer to the data sheets which were passed out (See Appendix B). Please refer to Table I. We picked five mills, both sulfite and kraft process, with various types of pulping techniques and various types of waste treatment. The American Can kraft mill (Halsey, Oregon) is what I think we could call the state-of-the-art in pulp mill waste treatment. There is a series of area lagoons for secondary settling of kraft process mill wastes, and the best equipment and engineering went into the plant. Likely, it is considerably overdesigned. We looked at the mill effluent and then came up with counts upstream and downstream.

I conducted a whole series of filtrations, picked individual colonies, and confirmed their ability to ferment lactose with production of gas, yielding confirmed total and fecal coliforms. We inoculated EC broth at 44.5°C, and fecal streptococcus KF agar. This number of *Klebsiella* was calculated based on a percentage of colonies identified as *Klebsiella* from a percentage of the colonies that were picked. If they were *Klebsiella*, I referred to the original count on the filter and got what I would consider a semi-quantitative value for *Klebsiella pneumoniae*. Criteria for identifying *Klebsiella* were --++ IMViC test, lack of motility, and the ability to decarboxylate ornithine. These results were confirmed by the National Communicable Disease Center (NCDC), so I am confident that the numbers are reasonably accurate; if anything, they are low.

Referring to the Crown-Zellarbach mill and the verified tests, note the extremely high coliform counts. The fecal coliform counts are also high. One thing that is worth noting on that page is the first value of 4,200,000/100ml I discovered that the waterbath was at 44.1°C, which was at that time within the limits of the test (that was 44.5 plus or minus 0.5°C). I then raised the temperature to 44.5; there was an apparent, significant drop. I suspect that the drop has to do with an increase in temperature of 0.4°C.

Note the high coliform, fecal streptococci, and *Klebsiella* counts for Crown-Zellarbach mill effluents and for the S. Santiam River. Upstream, there are a number of municipalities that are discharging treated wastes into the river. Coliform counts increased significantly, and there was a 9,000-fold increase in the concentration of *Klebsiella pneumoniae* in the area downstream receiving the effluents. The kraft mill at American Can Company consistently had low concentrations of all indicators. With a few exceptions, I could not isolate any *Klebsiella* there.

In attempting to isolate *Salmonella* out of these wastes, I took a liter of sample and filtered it through diatomaceous earth. I inoculated the "plug" into an enrichment medium and tried to get a qualitive *Salmonella* isolation. In all cases, including those of the effluent and receiving water, I failed. This doesn't mean *Salmonella* weren't there; it just means I couldn't get *Salmonella* as far as Im concerned.

Brezenski: Are the *Klebsiella* counts based upon total coliform plates?

Bauer: Correct.

Brezenski: What was your rule of thumb in terms of how many relative percentages of your columns checked out?

Bauer: I assumed that the colonies were layed down on a filter in a random manner. I would start at the upper, left-hand corner of the plate, pick everything that looked like a typical coliform until I hit 20 or 30 colonies, calculate the percentage on that figure, and apply it to the original number of colonies on the plate, then multiply that by the correct figure.

Gallagher: You're only measuring process wastes?

Bauer: Absolutely.

Vasconcelos: You said you had a portion of these checked? They were all confirmed by NCDC?

Bauer: I took the first 60 isolates and sent them to CDC. They came back 60 positives (*Klebsiella*). I'm convinced that the method used is, if anything, conservative. I understand, for instance, that M-Endo Broth is slightly inhibitory to *Klebsiella*, so some strains probably would not grow. If anything, those results are low. I also excluded any culture that was indole-positive, and approximately five percent of *Klebsiella* are; so again, these results are low.

Vasconcelos: Did they refer to this *Klebsiella* as Type I or a mixture?

Bauer: There are 77 serotypes of *Klebsiella*. They gave me a specific serotype where there was sufficient capsule to type. I can tell you right now that you'll get every kind of colony morphology in the world: smooth, rough, mucoid; on EMB agar you just cannot tell. There was no way I could look at the plate and say, "That is *Klebsiella*" If there was a great big mucoid colony, I was very suspicious, but I tried, at first, to differentiate smooth from rough; it was completely hopeless. The biochemicals were the only way I could tell.

Indicated in Table II are data that were prepared by Dr. Tom Aspertarde at Crown-Zellarbach. The total-coliform bacteria numbers indicate that either the MPN or the MF will give reasonably comparable data. Moving to Table III, I then attempted to do frequency distribution of the IMViC types from different isolates. Approximately 600 coliform and 300 fecal coliform colonies are represented by these statistics. In most cases, the --++ IMVic type is certainly predominant. With the exception of Crown-Zellarbach, and I didn't calculate a percentage of ++--IMViC types, they were negligible. I recall something on the order of three percent. I was not satisfied that I really had enough colonies in order to put down a number. That is what is represented in the column entitled, "all mills." Those numbers represent approximately 500 isolates. I just lumped them all together, 3.3 percent Type I *E coli*. Bear this in mind: those all came from Crown-Zellarbach. That was my particular experience.

From fecal-coliform bacteria plates [Table IV] the percentage producing these IMViC types, which are *Enterobacter* types, is even higher -- 89 percent at Publishers, 100 percent at American Can, 87 percent at Weyerhaeuser (a biological treatment process); Crown-Zellerbach somewhat lower. In this case for the fecal coliform, I came up with a healthy percentage of Type I *E coli.*

Then the next question arises: How many of these "aerogenes" IMViC types were *Klebsiella*? It appears that most of them are. Combined data, from all the mills (Table V), of all the "aerogenes" IMViC types, approximately 50 percent of everything, indicate 84 percent of these are *Klebsiella pneumoniae*; from the fecal-coliform bacteria tests, 92.5 percent.

Then, I compared frequency distribution of IMViC types from my data with some of the numbers that Ed Geldreich has in his publication "Significance of Fecal Coliforms in the Environment." Certainly, there is a significant difference in the IMViC type distribution (Table VI). The majority of them are *Enterobacter* IMViC types.

In Table VII is indicated, in the first column, a description of the colonies from a very minimal effort I made. These colonies are the ones that could be called fecal coliforms. The colonies were blue from edge to edge, cream color with a blue center, or cream color with a light purple center. The next columns (Table VII) indicate, respectively, the number of colonies picked, the percentage of those that were lactose-positive, the percentage of those that were subsequently positive at the elevated temperature, and the percentage of Type I *E coli.* It does appear that one can discriminate, at least to some extent, based on colony morphology, on the MF membrane. Although there is a small number of colonies, you will note the ones with the blue centers that are Type I *E coli.* I should like to show you a few pictures of the plates which I photographed. (I am sure you have all seen them a million times).

(Projection of color slides demonstrating total-and fecal-coliform bacteria colonies on the membrane filter).

That is the kind of problem that we frequently run into in industrial wastes. This was from Publishers Paper Company (Oregon City, Oregon), which is a magnafite-process sulfite mill. At that time primary settling was its type of treatment. Magnafite process involves a degree of chemical recovery. The BOD of this waste would not be as high as that of an ammonia-base sulfite mill. When you see something like that, you wonder what you count. They are not all that bad. Standard Methods says: "Do not count the gray colonies," and sometimes I am not sure I am seeing gray or blue.

Geldreich: You have an overloaded membrane to start with.

Bauer: Yes, let us move on to the next one, a little cleaner plate to count. This was Crown-Zellarbach (Lebanon) with 0.005 ml of inoculum. These colonies were blue. They photographed as black, but this M-FC medium with an agar base, a personal preference of mine, tends to make the colonies a little darker. This (pointing to slide) is the type of thing that I was calling blue center, somewhat mucoid in appearance. This is what I was calling blue, edge-to-edge. Let us try another slide. This is again Crown-Zellarbach (Lebanon). The total-coliform bacteria membrane shows a reasonably good sheen production. I should say that you would normally identify those as being coliforms. Number 37 was the mill waste itself, and this is the downstream sample, 38: the filtration volume was 50 ml.

Next, these cultures were streaked on EMB agar. This is a fairly typical plate (referring to slide). They are not all that way. Some will give a nice green sheen. There is every kind of colony morphology you can imagine. As I recall, Harold Jeter, National Training Center (Cincinnati, Ohio), counsels you on the completed tests to streak them out on EMB agar, turn the plates over, and look for a nucleated colony. Is that correct?

Geldreich: That is correct. There is one thing about EMB agar that is not very well understood by most microbiologists. The textbooks have never clarified EMB reactions. The differential characteristics of EMB agar are pretty poor. Sheen colonies may or may not be *E coli*. The fish-eye colony may or may not be "aerogenes." The literature or textbooks give you the impression that there is a sharp differentiation with this medium between *E coli* being a metallic sheen organism and *Enterobacter* looking like this. In this case it is probably *Klebsiella*.

Bauer: That is *Klebsiella*, by the way.

Geldreich: There is not that sharp a differentiation on EMB agar.

Bauer: I found every kind of IMViC type in the world, just by streaking out "aerogenes" types on the EMB medium. After a while you get a tremendous variety of colony morphology. One characteristic in common is they are all nucleated. I just streaked them out to purify the culture, by the way.

Geldreich: This EMB agar procedure is really nothing more than a way to isolate colonies, a process you are going to carry through to a completed test. There have been options made in Standard Methods to use

EBM or Endo agar. In our country the textbooks, Standard Methods, up until recently, (as well as Difco's or BBL's label), state that a colony is *E coli* if it produces a sheen colony on Endo agar. This is not true. Red colonies are something else again. Endo agar will produce a sheen colony, if it is properly made, but this is a misconception. When you sterilize Endo medium, you destroy its capability for giving the sheen production with all coliforms. What you are seeing is just a few of the coliforms with sheen colonies on Endo agar. If colonies are picked from the membrane filter and streaked on properly made Endo agar, all coliforms will have a sheen colony.

Riordan: You have percent "aerogenes" IMViC types, and then "aerogenes" identified as *Klebsiella pneumoniae*; where would *E coli* be represented in the data?

Bauer: In the frequency distribution tables that I referred to earlier, the ++-- types.

Riordan: My basic question then is, can you make any inference? With respect to the *E coli*,..can you say what percentage of the fecal coliform are *E coli*?

Bauer: I think it is not in that table you are looking at, but it is in this other data (Tables III and IV) under "all mills," indicating that 3 percent from a total coliform plate, and 15 percent from a fecal coliform plate are *E coli*. You can compare those with the different types of processes. The percentages will vary depending on the pulping process and the treatment type.

Riordan: Referring to earlier tables, could you break down the data from Crown-Zellerbach to get *E coli*?

Bauer: Yes, you could do that. This is one of the issues that we have to come to grips with. Can we use straight fecal-coliform bacteria tests, or is it necessary to go on to determine whether or not they are *E coli*, and can we base the standard on *E coli*? This is going to be a very controversial subject.

Stein: In addition to determining the kinds of organisms, did you attempt to correlate the information with other chemical data and the type of treatment?

Bauer: Yes. We measured the BOD, COD, and every kind of nutrient imaginable in the attempt to correlate them with the count, without success.

Stein: The notion is that no matter what type of treatment you are thinking of for the pulp and paper business--and the assumption is that the American Can plant is the optimum example of treatment today--we are not going to obtain a significant reduction.

Bauer: That is correct. American Can has a beautiful effluent.

Stein: No, I mean on the coliforms.

Bauer: The coliforms were uniformly very low, less than 1,000. In the final effluent, fecal coliform counts are, consistently, in the range of 20.

Stein: But there are some high numbers here.

Bauer: The percentage may be high, but the number is low.

Stein: Let us assume that they are going to ask all the paper mills to match the American Can standards. Will we, by that process, lower the coliforms to such a low level that we may not have to think in terms of disinfection?

Bauer: That is precisely what should be recommended. May I expound more as long as you have asked this question? Crown-Zellerbach had a microfermenter in that particular biological treatment system which, as you know, EPA subsidized, a situation that allows us to get in to sample it at any time. The system has approximately eight days of detention time in it. The counts in the intake waters are about 10^3 per 100 ml total coliforms, and they come out anywhere from 10^7 to 10^9. There is a lot of regrowth going on in the plant process. American Can puts this material in a microfermentor and lengthened the detention time to 18 days. The result: the total coliform count is 9,000. The effluent looks beautiful.

In contrast, the effluent at Crown-Zellerbach is so viscous and rancid that it might congeal mementarily. No more than 10 ml could pass through a membrane filter. One can biologically treat these wastes to low bacterial levels and, while doing that, one gets rid of the BOD. The material finally starts to settle. One of the biggest problems in biological treatment is the inability of the floc to settle, and the tremendous wastage of solids. Increase that detention time to 18 days and, naturally reduce the coliform levels to some reasonable level. The result is stabilized waste; so stabilized that it is not going to cause an added persistance of bacteria downstream from domestic wastes. If one increases the nutrient level in the stream, then the persistence of enteric bacteria in the receiving water will be increased. If one stabilizes that waste, the metabolizable carbohydrate is removed. There will not be any large re-growths of undesirable organisms that are potentially deleterious to life.

Stein: One can get these numbers down with a reasonable detention time, whatever the treatment is (one has the American Can or its equivalent, or one has your 18-day retention time, or physical-chemical treatment). Obviously, when one uses physical and chemical treatment, there will be a lot of resistance from the mill. Then, there is the other problem. Mills are either going to own the land and be able to give us the retention time, or with other mills, there will be a determined amount of resistance because there is no space. We can talk about 18 days, but in large companies, we are not going to get it. The question is: If one can get this treatment from American Can, below 1,000 fecal coliforms, should we require these companies to go below 1,000? Should we require them to do it any way they want to do it--retention, physical and chemical, or disinfection--or would it really make any difference? The point is this: If they are above 1,000, what over-all significance will that have?

Bauer: I would prefer to see treatment to the level of 1,000 used rather than to see disinfection employed. If the nutrient is still in the waste, those organisms that survive, oftentimes re-grow, returning to the level they were. One still has, in the waste, the unstabilized carbohydrate that is going to initiate re-growth in the stream, increase the persistance of enteric bacteria, etc. If it is at all possible I would recommend to biologically treat to a "reasonable" coliform level.

Stein: You're not saying biological?

Bauer: Well, no, physical chemical treatment. Polyelectrolytes work beautifully on these wastes.

Stein: But if the industries do not have the space to give us biological treatment, we should at least offer a counter proposal if we are interested in coliform reduction. It is not clear whether we should put a restriction on them. We have to answer the basic question: Is there any pathogen problem from pulp and paper wastes, however high the fecal or the general coliform count? If we answer that question, the industry may be faced with the problem of not enough land and with either putting in physical and chemical treatment or disinfection.

Unless I'm wrong, there may be some other problem here. The price difference may be so great that the companies may say, "If we're going to be forced to, we want to disinfect and we are not going to put in physical and chemical." If so, we should determine from a scientific point of view just what we should require. If we have massive resistance from industries in getting them to put in something directed specifically toward reducing the coliforms, we should have some very specific evidence of the dangers before we can ask them.

Bauer: I hope people subsequent to me can shed some light on that subject.

Lyons: From the engineering standpoint, when one is formulating the rules, one is considering several trade-offs in retention time, in microbiological concentrations, and in how they all affect the reaction rate. With 18-day retention time there is quite a decrease in rate of return as far as substrate removal is concerned because of less organisms. The whole thing is the cost trade-off between doing biological treatment or some form of physical and chemical treatment or disinfection.

No one is going to lock into it right now, but have these considerations been investigated?

Bauer: Along the engineering line, I am convinced that it is technically feasible to treat them physically, chemically, and biologically--some way to reduce those numbers without having to resort to disinfection.

Stein: It is our view that disinfection is not satisfactory, because of the re-growth. That has nice implications, for disinfection is what municipalities are doing along the Willamette River.

Geldreich: I have been giving Mr. Stein testimony for years that we have a problem in this same area: nutrient removal. A case in point if Wynopscot Bay. If the nutrients were lowered, this would then solve the problem. It is necessary to recognize, somewhere, what is the value of the downstream water use. Is that recognition going to be so important that it is going to demand that industry treat its waste in order to meet a certain limit? It may be shellfish we're talking about and that's....

Stein: That is another case.

Geldreich: Yes, but it is a case we have to recognize.

Stein: We shall come back to that specifically. I think that you get the point with all these economic questions. The industry would be much happier to spend money on nutrient removal or on removing the solids, than to be tagged with bacterial removal, which of course, may mean that they (the companies) are creating a public health hazard. If one can give the rationale in order to put this another way, where we are going to achieve this removal, and not really hit the industry head-on, then it will be a great advantage, because the companies are going to fight to the death to disprove that there are health hazards. If we can achieve these reductions with other benefits to the stream, this method is going to be the more acceptable one.

Geldreich: We recognize this in the sugar beet industry. In the reports, we are saying that if the industry achieved nutrient reduction, say 85-90 percent BOD removal, in that area, we would very well accomplish acceptable treatment.

Bauer: The State of Oregon requires coliform data from all the municipalities, and the municipalities have considerable amounts of correlative data with suspended solids, BOD, etc. If one measures the one parameter, the coliform, as a monitoring tool, then a lot of inferences from that can be made. Oregon has found, with municipal waste, that if the total coliform value in the effluent is less than 1,000, and if municipalities use a very high level of chlorination (1 mg residual after 60 minutes detention time) the result is excellent correlation with good treatment efficiency. Suspended solids are uniformly below 20 mg/l; BOD is uniformly below 20 mg/l. "Since I am in the surveillance business now I am looking for tools like this. If I can tell our Permit Compliance Division that if we look at coliforms and they are at a certain level, I can give you reasonable assurance that the waste is well stabilized--BOD is within this range, etc., we have a tool."

Stein: On the Missouri River it was unsatisfactory if they treated wastes or if they didn't treat the wastes. When regrowth occurred it has to be recognized that if we chlorinate and disinfect the wastes, and if we are satisfied with what is being discharged from the outfall coming out of that pipe, the effluent may not be enough to protect the stream downstream if regrowth occurs and something else happens.

I am trying to indicate one of the significant problems: *Just giving us a number, say X number of coliforms, after the waste comes out of the effluent pipe, may or may not solve our problems. You have to indicate to us a method and you have to specify a way of reducing the nutrients, whether the preferred way is a retention time or a physical-chemical method rather than disinfection.* This is something we should know about because we may require a company to spend several million dollars and then we have to come back in a year or two because that company has not cleaned up the stream. This should be the philosophy we have to follow.

Again, let me make this last point because it is a big one. Suppose we ask companies to put in a complete system, ie, similar to that of American Can, and suppose their fecal count is higher than the EPA limit so that the company is in violation. The firm says: "OK, now you (EPA) tell us what to do." EPA is obligated to tell them something, and something with some meaning. Maybe it is to be disinfection. Now, if the policy suggested does not have any meaning after EPA has asked for the treatment equivalent to that of American Can, there are going to be more problems. Over the next few years as these better mills go into operation, those are the alternatives.

Very soon it will be necessary to make a determination as to whether we are going to ask for any kind of restriction in terms of bacteriological parameters, particularly in canning and sugar beet plants and in several other industries. We have to assume that we will have to require a certain amount of BOD and solids removal. The question is: *If we are going to ask for removal, can we forget the coliforms? Can we ask for a complete treatment system, including nutrient removal and disinfection? What is the BOD of the American Can Company, for example?*

Riordan: With reference to American Can, what are they doing in terms of BOD and suspended solids removal? What have we got, 10 pounds per ton production-could it be less than that? I think the company wants 6 to 8 pounds per ton production at 30 mgl BOD in terms of effluent.

Knittel: In contrast--something which may come up later for discussion. We are trying to set American Can up as being the ultimate in removal, and, especially, we are addressing ourselves to the aspect of coliform removal. The firm is a relatively new mill (approximately two years) whereas the other mills that we have been talking about, during the date presentation, are older, well-established mills. These older, established mills, over a period of time, have probably become colonized with coliform organisms and they do not represent merely domestic sewage contribution. What we need to do is to keep looking at American Can from a certain standpoint: Do the processes become colonized? The waters that they are using are fairly high in coliform organisms. Over a period of time the system is going to look and act somewhat like a continuous culture.

Lyons: To get back to Mr. Stein's point, what do we do initially?

We have about two weeks to come up with some reasonable starting point. Now, let us understand that this is not going to be hard and fast; instead, it is going to be a reasonable working point--as were the other limitations that were pointed out.

Bauer: Could we hold the discussions, please, until we get through all the presentations and then we can start arguing about it.

The second handout (Appendix B) is a collection of analyses on a variety of waste sources. The National Council has become convinced that the only bacteriological parameter of sanitary significance is *E coli*, so we screened all these different places for the presence of EC positive cultures (Table VIII).

Resi: Yes, but these are natural "elevated temperature bacteria."

Bauer: Right. In fact, the Council will not call them fecal coliforms. The National Council refers to them as "elevated temperature coliforms." Tables IX and X show some data that were recently obtained out of the Snake River. Included are both effluent data, and data from the receiving water, by the total coliform, fecal coliform, and fecal streptococci tests. These are not verified counts. (The normal Standard Methods membrane filter technique was used).

A form of bacteriological mass balance was performed on all these numbers. Known quantities were the flow in the river, and the contribution of all the municipalities and industries; we computed the expected concentration of coliforms. If one assumes complete mixing from all these various sources, the predicted coliform concentration in the Snake

River is about 11,000. Obviously, from some of these numbers in the receiving waters, the counts are considerably higher than that. Note, for example, the 260,000 average below the Idaho Falls sewage treatment plant. You will notice, on these receiving waters, that the arithmetic average for total coliforms in the upper Snake River is well within a recreational water quality standard.

Downstream further, around Burley, Idaho, the regrowth of total coliforms is very apparent. The averages were several hundred thousand. There is no way to account for the presence of all these coliforms except as a regrowth phenomenon.

In Table XI, I played the game with the frequency distribution of IMViC types again, grouping together the various types of treatment plants with food processing, meat processing, etc. With the exception of fruit-packing plants, *E coli* were present in all of them.

Brezenski: Would you summarize your presentation, Mr. Bauer?

Bauer: In summary, I think that some of the contentions of the National Council that no *E coli* are present are not completely accurate. The percentages are low, but nevertheless, in most cases the bacteria are there. (I think that Dr. Martin Knittel may be able to corroborate that later). *Klebsiella pneumoniae* is present in sulfite mills, consistently, in very large numbers. Whether or not that represents a potential health hazard I shall leave up to the medical doctors, the epidemiologists, and so forth. These organisms will regrow quite readily if you attempt to disinfect them, unless the BOD is reduced significantly. I am convinced that methods are available to either biologically or physical-chemically treat all of these various types of industrial wastes to achieve a low bacterial content. In my own opinion this is desirable. I would prefer treatment without having to go to disinfection. I have not found *Salmonella* in any of these wastes. Some of the other people here may or may not have found these organisms. The National Council says no bacteriological criteria should be applied to their effluents. Are there any questions?

Brezenski: In general, you are telling us that on the whole, your beliefs are compatible with what the NCASI states except that the Center is saying it is not passing *E. coli* in their effluents.

Bauer: My opinion is not compatible with theirs, to the extent that I think that no bacteriological criteria should be applied to their wastes.

Brezenski: No, looking at the data, not looking at the Council's conclusions.

Bauer: But that is the conclusion they come to. The inference, if you say I agree, it is that I agree with their conclusions, and positively, I do not.

Riordan: Why is there so much dependence on *E coli?* Are not *Enterobacter* also found in the human intestine?

Bauer: Yes. *Klebsiella pneumoniae* are also present in the human intestine; 20 to 30 percent of the people are carrying it all the time.

Riordan: Is *E coli* more specific? If that is a much better indicator, is that why?

Bauer: Uniformly, it can be found in the gut of warm-blooded animals. There is a percentage of humans who do not carry *Klebsiella.*

Klebsiella, Enterobacter, Serratia, etc. are found in the environment, in decaying vegetation, and in many sources, although the concentrations are not particularly high.

Lyons: You said significant BOD reduction; what level are you talking about?

Bauer: The waste at Crown-Zellerbach goes in at 800 and comes out 300; that is not adequate. What is adequate, is as yet unknown.

Lyons: Are we going for percent removal?

Bauer: I would prefer not using percent removal.

Lyons: I would just like some rationale on which to set effluent quality, because if we chose a quality of 30 mg/l BOD, is this a level that will greatly inhibit this regrowth?

Bauer: I can live with that; I do not know about other people. That figure is certainly a vast improvement over the proposed policy passed around today.

Lyons: I would like to comment on that. I wrote that permit proposal based upon only my sanitary engineering background and about three hours of microbiology. It was not intended as a final document, but to be a basis for discussion on what can be written into permit standards. The concentration is about 30 mg/l, using statistical flows and unit operations. This sounds good except that I am wondering if sulfite mills will be considerably higher because of the status of treatment technology as applied to this particular waste. You said, too, that you restricted the presence of *Klebsiella* to sulfite wastes.

Bauer: No, we are looking at kraft also. It is present there but in low concentrations. The basic difference is related to the type of sugars that are left. One has chemical recovery in the kraft, and the sulfite process contains up to 15 percent pentosans, pentose-type sugars. Likely they are responsible for the preponderance of that particular type of organism. *Klebsiella* can use pentoses, quite readily with a large variety of enzymes. In the kraft process wastes there is not nearly that level of sugar available.

Geldreich: With high nutrient waste of this type, 30-60 percent removal is relatively poor.

Speaker from the floor: The elevated temperatures used in these processes and the high bacterial populations certainly emphasize the need for monitoring these wastes.

Geldreich: I think the figure of 30 mg/l is reasonable, looking at it from the research point of view. Some of the experiments that we or others have done with fecal coliforms in highly treated sewage effluents indicate that 14 to 30 mg/l could very well control this problem of nutrients, if we have to measure it by something as crude as the BOD. Temperature certainly is important, but if you can throw a block in one of these things--and these would be nutrients--the temperature in the summertime would not be too much of a problem to overrun what one is trying to do with nutrient removal. This makes more sense than our older approach to the problem i.e. asking for 85 to 90 percent BOD removal.

Lyons: This approach makes more sense, if it is technically attainable with the type of treatment available today, than to call for percent removal.

Geldreich: Yes. What we are trying to remove here, in terms of the nutrients--so those of the engineering group can understand-- are the nitrogen and carbon sources. Throughout this discussion we are not talking about phosphates and nitrates, but about carbon and nitrogen sources. These nutrients and temperatures, as well as the bacteriological population, are very critical items to control.

Lyons: Actually, we are trying to control nitrogen and the combination of materials that are in our treatment plants, and we would just as soon have a bacterial monitor to do the work.

Geldreich: For a monitor, one needs a bacteriological measurement to back up what your chemical parameters indicate. Two systems are available to support a contention that this would do it.

Bauer: One of the issues Mr. Stein raised was: Are there other things in these wastes that might be pathogenic? Therefore, I asked Don Herman from National Water Quality Laboratory (Duluth) to go through the data that has been working up during the last five or six years and try to give us some insight into that.

Experiences with Coliform and Enteric Organism Isolation from Industrial Wastes

Donald L. Herman

A lot of the industrial material which I shall be discussing was collected prior to my affiliation with FPA except for the data from the paper and pulp mills and domestic secondary effluents. These few slides from an area in Minnesota will demonstrate the problem.

(A series of slides depicted obviously high quality stream water becoming progressively more polluted downstream. Areas below the entry of pulp and paper mill wastes were shown. Recreation use was illustrated).

This stream headwaters in the Superior Forests. During an entire summer we found a positive coliform count only from five to a dozen times. One may find one fecal coliform count during the summer. This water is very low in nutrients. As we go on downstream we observe the effects of pollution. Some of this nutrient addition is due to the pulp and paper industry. There is some water where we have complete dissolved oxygen loss. Then there are some fish kills. Then down close to the communities involved in various discharges, the river is merely devoid of any form of life. On downstream we find floating material and sludge development. We are involved, in most cases, with some recreational uses at one time or another--fishing, swimming, and bathing.

(Slides show total and fecal coliform colonies on the membrane filter).

These slides show the coliform colonies on the membrane and the type of colonies we find. The fecal coliform membrane illustrates the type of colony morphology. I will discuss this later. We picked any colony that had any blue to it at all. Very often if we incubated these plates a little longer, colonies picked up the blue color regardless of morphology. As far as *Klebsiella* goes, I think we have to consider reduced metabolism of the lactose. The color addition results from metabolism of that type of sugar. Here we made an isolation from EMB agar and this one is *Klebsiella*. We found various types of morphology. It shows some of the encapsulation you get around the cell.

I now refer to the handout sheets, page 7 in Appendix C. These data were collected--before I became associated with EPA--when I was working with the canning and food processing industry. Most of the work was with treatment lagoons. During the initial treatment stages of aerated lagoons in this industry, the BOD was approximately 1400 mg/l; our range was 160-2400 mg/l. These data represent three different plants. The survey extended over a two-year period. Flow rate averaged about 125,000 gpd. The total coliform bacteria level was 320,000/100 ml, ranging from 2,000 to 1.5 million/100 ml. The fecal coliform was 15,000/100 ml and the range was 100 to 60,00/100 ml.

The fecal coliform work was performed using the MF and the *Salmonella* work was with the MF on XLD medium. We tested *E coli* Type I because most of the states we worked with did not care about *E coli* but wanted to know what we had in *E coli* Type I. These effluents had 35 percent Type I. *Klebsiella pneumoniae* in any work we do is a nonmotile organism and we key it on down to the *pneumoniae* organism because other species of *Klebsiella* are involved in animal infections.

The *Pectobacterium* was 6 percent. *Salmonella* species were running 0.7 percent. Now after post treatment, and this is after some twenty-one days, the total coliform density was 3,000, the fecal coliform density 140/100 ml. *E coli* was running 32.1 percent, *Klebsiella pneumoniae* 42.9 percent, *Enterobacter* species 5 percent, *Pectobacterium* 9.3 percent, and in this case we had an increase in the percent that now is going out, *Salmonella* 10.7 percent.

Bauer: Don, that's percent of what, samples analyzed?

Herman: No, in this case, I refer back to the fecal coliform factor there, 140.

Bauer: How about the *Salmonella* species? .7 percent of all the samples?

Herman: Yes.

Bauer: That's a qualitative result?

Herman: Yes.

Resi: One point here - I do not think this is the best way to recover *Salmonella*; therefore, I think this point should be made.

Herman: I was just going to get to that.

Bauer: But, you can conclude that they were there in a certain percentage.

Herman: In the first few samples we could not even find a pair, then we got into some bottom samples. In these cases you can go to the stream bottom and find if the organisms are there. We ran XLD, SS, Tetrathionate and various combinations of media to find them. On page eight are carbonated and non-carbonated beverage waste data. We got very high reducing sugar levels here. The initial BOD was 1600 mg/l and we had a range of 800 to 18,000 mg/l. The flow was not too high, about 36,000 gpd. Total coliforms were 7,500,000/100 ml; fecal coliform, 50,000/100 ml. The types of isolation were: *E coli* Type I, fairly low, 5.6 percent; *Klebsiella pneumoniae* 68 percent; *Enterobacter* 15 percent; *Pectobacterium* 7.0 percent, and *Salmonella* 4.4 percent. On post treatment, the persistence seemed fairly high on some of them.

I will proceed to discuss potato wastes. On the initial stage, BOD was approximately 14,000 mg/l and the range of 1500 to 25,000 mg/l. Flow rate was

75,000 gpd. The total coliform bacteria was 160,000,000/100 ml; the fecal coliforms, 265,000/100 ml, and in a range of 18,000 to 850,000/100 ml. In this area, *E coli* type I was only 0.9 percent; *Klebsiella pneumoniae* 81.1 percent; *Enterobacteria* 9.4 percent; *Pectobacterium* 6.9 percent; and *Salmonella* species were 1.6 percent.

Bauer: How many samples did you process to achieve this *Salmonella* species percentage?

Herman: I would have to go back over my figures on that topic. Put it this way: There is not any waste reported here that involves less than 200 samples.

Bauer: O.K.

Herman: Regarding isolations, we have about 25,000--all types of isolations. Most of them were recovered in a period of about two years. On post treatment the total coliform was 1700/100 ml; fecal coliforms 45/100 ml. Isolations gave *E coli* Type I of 15.5; *Klebsiella pneumoniae* 60.1 percent; *Enterobacter* 8.9 percent; and *Salmonella* at 15.5 percent. Note the shift of what is represented within these ratios. We had confirmations back from NCDC on the *Salmonella typhimurium* and the *S. St. Paul.*

Meat and slaughter wastes were just loaded (page ten). Initial BOD was 26,000 mg/1; flow rate, 28,000 gpd; and total and fecal coliform 670 and 6.5 million/100 ml respectively. Results of the isolations were: *E coli* type I of 56.9 percent; *Klebsiella pneumoniae* 21.5 percent; *Enterobacter* 13.8 percent; *Pectobacterium* 0.5 percent; and *Salmonella* was at 7.3 percent.

After treatment, the BOD was reduced to 400 mg/1, a 98.5 percent reduction. That is quite a high BOD for a discharge to receiving waters. The retention time in the lagoon was thirty six days. Total coliforms were 250,000/100 ml and fecal coliform 18,500/100 ml. Isolations gave: *E coli* 65.8 percent; *Klebsiella pneumoniae* 24.1 percent; *Enterobacter* 6.9 percent; *Pectobacterium* 0.9 percent; and *Salmonella* at 2.3 percent. Initial random isolates confirmed as *Salmonella typhimurium* and *S. St. Paul.* Resampling--because there was a question on these results--confirmed these findings, and added the variety *S. copenhagen* as well as *Shigella sonnei* and *S. flexneri.*

There were firm orders, from that time on, that one really did not need any more sampling of this type. All that was required for both Federal and State tests were total coliform counts; that was all that the regulatory agencies were going to get.

Now, I refer to the paper and pulp wastes. This phase of work resulted in some real challenges and the utmost use of tact and diplomacy in order to obtain valid samples. It is still a question of whether the samples represented full production stress of the plants that were involved. The initial state was aerated lagoons. They never would tell me the flow rate.

Question from the floor: What kind of mill was that, kraft or sulfite?

Herman: Two of these are sulfite and one is kraft.

The initial BOD was about 2200 mg/1; total and fecal coliforms 1.5 million and 8,000/100ml respectively. We made initial checks; the correlation work was carried out by the State, and indicated that this waste was extremely devoid of nitrogen content; in other words we were looking at nitrogen. *E coli* Type I was 4.4 percent; *Klebsiella pneumoniae* 85; *Enterobacter* species 9.5; *Pectobacterium* 0.8, and *Salmonella* 0.3 percent, and we could only get *Salmonella* when we got to the interface sample. If we took a sample out at any other part of the lagoon we did not find *Salmonella*. For post treatment discharge without chlorination, BOD was about 3,600 mg/1. Retention time was two days in the lagoon.

The total coliform bacteria density was 57 million/100 ml and fecal coliform ran 130,000/100 ml; *E coli* Type I was 0.4 percent; *Klebsiella pneumoniae* 92.3; *Enterobacter* species 6.7; *Pectobacterium* 0.6; and *Salmonella* species at 0.0008 percent. There were more than 200 samples.

For a lot of these wastes, one is talking about nitrogen deficiency. A number of these organisms, especially *Klebsiella* employ the nitrogen from air--a selective medium is created. There is no reason why one should not have some selection of the microflora response to these various kinds of wastes.

We did conduct some additional field survey work on the receiving rivers. One river demonstrated fish kills due to lower DO in 1969 and 1970. The CODs in the river ranged from 12 to 260. That picture of the dead fish on all that high quality water was taken during that period when we were out in the boat getting samples. Total coliforms ranged from 10 to 171,000/100 ml and fecal coliforms from 3 to 75,000/100 ml. Fecal streptococci /100 ml ranged from 6 to 1350 and, in the course of events, eleven types of pathogenic bacteria were found and confirmed by CDC including: *Klebsiella pneumoniae, Shigella sonnei, Salmonella typhimurium, Staphylococcus aureus, E. coli,* and *Pseudomenas aeruginosa.*

We found one isolate of *Klebsiella* pneumoniae among these areas of major discharge while recovering types 14, 58, 21, 22, 31, 33, 35, 65, 7, 56, and 67, up to and including twenty seven river miles below the outfalls.

Within the river we found active colonized zones of *Klebsiella pneumoniae*, types 14, 15, 21, 22, and 31. These types were tested for mouse pathogenicity and were shown to be positive. Furthermore, in this particular area, these types and some similar ones that we obtained from clinical patients were analyzed biochemically, and were also sent to CDC for typing. We exchanged cultures with Dr. John Matson, University of Minnesota School of Medicine. Clinically, we could not tell the difference between the ones recovered from fatalities in patients versus the ones we isolated out of the river. We got mouse fatality titers of 10^4 and 10^5 on patient strains; on the river strains it took titers of 10^6 to 10^7 to achieve the same effect on mice. Again, in the river, in these zones of colonization we picked up bottom rocks where you could see the cultures just growing right on them. These were taken to the Minnesota State Board of Health. Quite a few of them turned out to be pure cultures of *Klebsiella pneumoniae* growing on these surface edges.

The chemistry factors of interest were, of course, the decreased dissolved oxygen. We had none for some seven miles. Also, we found that as the pH decreased in the receiving water the more the enteric organisms there were present. There was a presence of reducing and hexose sugars; this was found for the entire twenty-seven-mile stretch. We found *Salmonella* once when fecal coliforms were 3/100 ml and continuously when the fecal coliform was more than 40/100 ml. The fecal coliform-to-fecal streptococcus ratio was averaging 1:14.6. Then, on downstream the ratio started to be 325:1. Bottom samples downstream from that area of discharge ranged from 50,000 to 5,600,000 total coliforms /100 ml. Fecal coliform bacteria were from 300 to 23,000 /100ml. In all bottom samples *E coli* Type I, *Klebsiella pneumoniae*, and *Salmonella typhimurium* were demonstrated.

For comparison, we ask, "What does this mean for some other areas?" In connection with some disinfection work we have some similar data on secondary effluents. We have disinfected with ozone, chlorinated, and dechlorinated, and then we followed through on the raw effluent. (These data are recorded over 2 1/2 years and 14,000 isolations). Total coliforms per 100 ml were 581,000; fecal coliforms 32,500. The *E coli* Type I was 62 percent, *Klebsiella pneumoniae* was 18 percent. Dr. Matson and co-workers report that, in cases of urinary tract infections over a ten-year study, that *Klebsiella pneumoniae* represent 18.6 and 18.7 percent of the total urinary tract infection from humans. Are we talking about a population factor being shown through here? It's curious. *Pectobacterium* was 3.6 percent and *Salmonella* species 2.1 percent.

Tests were conducted for *Pseudomonas aeruginosa*. We found these extremely difficult as compared to the other tests. The findings ranged from3/100 ml to 6,700/100 ml. The bacteria were highest in the meat and slaughter wastes. This area became a major workload to isolation and to confirmation with a limited staff. We tried Hektoen agar, which is suggested by King in a 1968 publication. Many times the results were open to question--especially when we got into higher quality water, as to what the colonies really indicated. Finally, we had to pick every colony on the plate. We found *Pseudomonas aeruginosa* present in low numbers where fecal coliforms were not detected. We also found fecal coliforms and *Salmonella* when we could not find *Pseudomanas aeruginosa*. I agree with the concern related to the organism, but prefer to think that it is part of the total picture of the microflora and not just the only indicator.

I have the results covering two pages from antibiotic sensitivity analyses on randomly selected *Klebsiella pneumoniae* from the secondary effluent, raw, chlorinated, ozone, dechlorinated, and back to the secondary. Then we have one from the Lake Superior and the Duluth water intake; the remaining were isolates made by Dick Bauer. We were informed by certain individuals, and especially the National Council, that all *Klebsiella* isolated from their wastes were not resistant to any antibiotics. There were no factors involved. So I decided to take a look at this. In summary, we will find that almost all of the *Klebsiella* are resistant to ampicillin. Some articles state that all *Klebsiella* should be sensitive to a Cephalothin. Clinically we have found this to be the case. These result from the environmental samples. From the clinical viewpoint angle we find that about 39 percent of the *Klebsiella* are resistant to that antibiotic. We have the strongest antibiotic resistance from the isolates from the paper and pulp wastes. We have one isolation of *Klebsiella* Type 33 that is resistant to every antibiotic available. In checking with the local physicians, we find there are a number of reported intestinal tract problems in the summer. Again, these are secondary infections and often not reported. When we reviewed the CDC reports, they confirmed the same thing.

To summarize quickly, my own experience indicates there may be a relationship between the bacterial flora of receiving waters and the flora of the intestinal tract, and to their behavior toward nutrients. In addition, too much nutrient in the substrate can totally disrupt the normal human, natural flora balance. The high quality of water we sampled was found to be extremely low in total coliforms and devoid, in many areas, of fecal coliforms--except downstream from a beaver pond or shore birds, and so forth. It just depends upon what other substrate is there. The solution has been pointed out in expert investigations by Gallagher, Anderson, and our FWPCA reports, the reports from the Red River of the North--they are all talking about nutrients and waste addition.

From working with industry, I know that the technology is available for better reducing of their wastes. If industry has the land available and makes use of the more modern waste reduction techniques, this can be accomplished.

Review of the *E coli* isolations revealed that 97.2 percent of these are typical fecal coliforms, *E coli* Type I. In all cases where the fecal coliform counts were greater than 100/100 ml, *Salmonella* was recovered. In 1972, Eickhoff, in his report to the Council, emphasizes the critical need for epidemiological work on the *Klebsiella pneumoniae* in relation to recreational waters. A proposal of this type was recommended in 1971, by Dr. John Matson. At last report, this proposal was completely lost in Washington. For developing legal actions, we certainly will need additional bacterial identification within these coliform counts. They are no longer going to accept what we say ("We have found so many coliforms"), instead they want to know which specific organisms are found.

In conclusion, as microbiologists, we must become well organized, work closely with the chemists and biologists, engineers, administration, and fellow microbiologists. I hope more meetings of this type are forthcoming.

Geldreich: May I ask what your explanation of the question: Why did we have more resistance of *Klebsiellas* to antibiotic sensitivities in paper mill wastes? I can see that doctors up in your area must be using the shotgun technique, with gantricin, to treat all their patients, i.e. a broad spectrum approach, but why it is we have so much resistance in paper mill wastes? Is there a logical explanation?

Herman: I hope some data on the nutrients and trace elements in this waste can come to light. On antibiotic resistance, in Anderson's doctoral thesis, it was strongly emphasized that, in the presence of sodium maganese and magnesium, there was a direct relationship to the ability of the organism to develop stronger resistance to antibiotics. My question is, do we have these trace elements in this type waste?

Bauer; That is a magnifite process where those isolates come from. Magnesium oxide is involved. Another possibility is that that particular mill is located downstream from a number of municipal discharges.

Herman: Some work by Buck shows strongly that in the laboratory recovery of *Klebsiella* the encapsulation factor is important. If the right carbohydrate nutrients are available, the capsulation becomes very thick and mucoid. So, we are talking about nutrient conditions again.

Berg: Was not the question one of difference between antibiotic sensitivity of free-living forms with that from those coming from patients?

Brezenski: I think there is another factor here that you are forgetting about completely; it is the R transfer factor.

Herman: This is another factor, and please do not forget this area.

We have strains of *Klebsiella* Type 33, from hospital patients who are resistant to every antibiotic. We also have isolates of *Klebsiella* Type 33 from recreational areas that are resistant to every antibiotic that we have. Review the literature on the R transfer. There are a number of articles on the transfer of antibiotic resistance from *Klebsiella* to *E coli* to *Salmonella*. In work by the Council, *Klebsiella* is found everywhere. The Council claims it found it on lettuce up to 10^4/100 ml. We analyzed a number of samples of cabbage (from the grocery store) and, out of some 200 samples, we got one *Klebsiella* isolation. Although it is found in animal infections, it is usually not *Klebsiella pneumoniae*. It would not be a surprise to find it there because *Klebsiella pneumoniae* is considered to be a part of the normal flora of the intestinal tract.

Bauer: Some of the participants at this meeting have asked what was discussed at the Corvallis Seminar. I will try to summarize. The National Council has been spending large quantities of money for admittedly defensive research, if they call it that, in order to try to discredit the fecal coliform test. They had some data that if they are accurate, taken on face value, are quite damaging. They would indicate that perhaps the fecal coliform test is not applicable to industrial wastes. You have heard very briefly, reference to some comments made by Dr. Razzel. He used to be with the pulp and paper industry in British Columbia and is a member of the ad-hoc committee. He and a technician went up into the headwaters of a watershed in Vancouver, British Columbia, and took samples of moss and various pieces of vegetation and then conducted fecal coliform tests on them. He spoke in generalities and did not give us any hard data, but he alluded to the fact that they found EC positive *Klebsiella pneumoniae* in the order of 10^6 per gram. These are rather surprising figures in light of the data that have been published by Bob Bordner, Ed Geldreich, and others.

They did this same thing with some of the wastes from the wood products industry in Canada. Essentially, the findings were the same. The one statement that was his real psychological cruncher was that they went down to the market in Vancouver and randomly picked a series of leafy vegetables and root crops. They carried out fecal coliforms to the *Klebsiella* identification, including EC. His statement that at any time you eat a head of lettuce, and at any time you have a salad in Vancouver, British Columbia, you're eating at least 40,000 EC positive *Klebsiella*.

All of the industry representatives said, "Hurray, hurray, we've got 'em whipped." They went off with big smiles on their faces and we did not have any data to refute it, at least not at that particular moment; that was a frustrating experience.

Geldreich: You do not need to refute it, Dick. Data are in the literature not only from our group. Bob Bordner and I have put out a paper summarizing much of the work of the South Platte irrigation water study. The data are not only on the South Platte but also cover areas where I had been involved within other areas in Utah and Wyoming, and Lou Resi also contributed data from certain areas, such as Snake River and a few other places in the lower Colorado River. Plenty of data, not only from our own organization, are available. There is plenty of data in the literature from the English, and also from Greek research that is being done.

Bauer: Unfortunately, I wasn't sharp enough nor was anyone else at that meeting to really hit them with it. I was very skeptical, but I could not quote anything off the top of my head. It made us look pretty bad.

Geldreich: I was going to ask you Dick; the vegetables they were talking about, were they actually grown in Vancouver?

Bauer: I don't know. It was never stated.

Geldreich: You know I was wondering. If they came from Mexico, they would be loaded. Was the study done in the winter? Obviously, the vegetables were not grown in Vancouver, but it would have been interesting to know more.

Bauer: I mentioned to you that these people were going to deliver this coup de grace at the last American Society for Microbiology meeting. I was hoping that someone here would have been sitting in on that particular presentation.

Vasconcelos: It was the worst session I had ever been to. It was just before lunch and it was packed, right next to the lunch counter. It was an open room and everybody was eating and I could not hear a word they were saying. No one else could either. I don't think there were any questions for that reason.

Bauer: I think that's the worst thing that could have possibly happened. That will get into the record without being contested.

Geldreich: Another problem Dick, is that apparently they failed to recognize, when they were discussing their results with you in that other meeting, that we do not live in an environment which is sterile in terms of not discovering some fecal contamination. In the remote areas we have a very high animal population about which people quite often forget. We have rodents in remote sections. In some areas deer populations are high. These people who are reporting this material are failing to recognize, intentionally or otherwise, that animal populations could very well contribute something to the particular site. They may have actually gone to an area and sampled where they know these animials are going to be coming to a water hole, or in an area where bacteria will colonize. There certainly are reasons why you can occasionally find them.

Bauer: I think that we probably could have refuted every one of Dr. Razzel's findings, but at that moment the rebuttal was not at hand. He and his associates left a very definite psychological impression which I hope that perhaps we can reverse today.

Our next speaker is Kathleen Shimmin from Region IX.

Observations of Selected Waste Discharges from Region IX.

Kathleen Shimmin

I would like to talk to you about some of the recent work that we are doing. When Bill Stang called and told me that this presentation was going to be forthcoming, we were in the process of verifying some permits of discharges in the San Francisco region. There are data being passed around right now that I am going to be talking from (Appendix D). You will see that it is of a very limited nature compared with Mr. Bauer's data or Mr. Herman's three-or-four-year observations. But, still it speaks to the topic that we're talking about today, and so I would like to present it to you. There are two main sections. One of them covers the data that were collected from sugar refinery and pulp mill discharges in the San Francisco bay area. The second is data that were collected in 1968 from a kelp processing plant in San Diego.

The data that you have here are gathered from total and fecal coliform determinations by the membrane filter technique. The samples were processed within three hours of collection. Colonies were then picked from the membrane filters, streaked on EMB agar to isolate them, and transferred to nutrient agar slants. They were then inoculated into Enterotubes, EC broth, and Brilliant Green Bile broth.

The first page (Table I, Appendix D) is identification of selected total-coliform bacteria colonies that appeared to be metallic green on the M-Endo plates. We have indicated the numbers of colonies and the numbers of types that were found by Enterotube identification. You will see for the sugar wastes, that primarily the total coliforms were of the *Klebsiella-Enterobacter* group. These were very low in positives, as revealed by the EC tests. All of them were confirmed in Brilliant Green.

In the pulp mill process water, the same outline was observed. The total counts were as indicated: 8,000-11,000/100 ml for the sugar wastes, 23,000 total coliforms/100 ml, and greater than 60,000/100 ml fecal coliforms by appearance on M-FC plates. For the fecal coliform colonies, needless to say, that was the surprising result.

We looked then at selected colonies from the fecal coliform plates. There we found that for the sugar processing plant wastes, the colony appearance was primarily gray-white colonies and of these, the majority were the *Klebsiella* type. We looked at just a very small number of blue colonies. As you can see in the sugar plant these were primarily of the

Klebsiella Enterobacter type also. In the pulp processing water, pink colonies were distributed across the board as far as the Enterotube identification went. A large proporation were of the non-*Enterobacteraceae* type. The blue colonies in the process water from the pulp mill were, also, a large majority of non-*Enterobacteraceae* type, although they did turn out positive so far as the brilliant green went. I think the difference between those two findings is that the Enterotube is incubated for twenty-four hours and the brilliant green, forty-eight hours. We picked up some slow lactose fermenters. (If you want to compare the counts they are listed on Table II.)

The conclusions that we made after viewing that data were that the pulp mill blue colonies were, primarily, not fecal coliforms, as would be identified by Standard Methods. Eighty-five percent of the blue colonies were not fecal coliforms. In the sugar wastes 90 percent of the gray-white colonies on the M-FC filters were the *Klebsiella* group; only 3 percent of these were EC positive. In the same samples, again very limited samples, 85 percent of the blue colonies were typical coliform type, but were not positive in EC broth. Ninety percent of the total coliforms found both in sugar and pulp wastes were from the *Klebsiella-Enterobacter* group. We have merely summarized the 1968 data. The plant that was investigated was one that processed kelp for the purpose of making alginate salts. The kelp process is one that would encourage the growth of anything in the kelp. It was steeped in warm water, at about 35°C, for up to about five days in this process; then the water was discharged into the bay. There were some studies performed in which the investigators found a very high coliform count, 32,000,000/100 ml. The count was also high for fecal coliform, an average of 580,000,/100 ml. Fecal streptococci were very low. Of the EC positive isolates, many were not typical *E coli*. We found 19 percent of the EC positive isolates were *E. coli* Type I; 17 percent was *E coli* Type II, and 74 percent not *E coli*, based on thirty-six different isolations. Many of the M-FC positive colonies were also EC negative. Seventeen percent of the M-FC positive colonies were EC positive and 83 percent, negative.

The conclusions that we came to after reviewing these data are that if we continue to use fecal coliform by MF as a parameter, we are either going to have to put in an extra step in order to verify the colonies or we shall have to make the medium more specific. Secondly, we certainly should consider choosing different parameters.

Geldreich: What do you propose?

Shimmin: *Klebsiella* might be a good possibility.

Herman: What did you give as your definition of *E coli* Type II, Miss Shimmin? Many people are confused when we speak of *E coli* type.

Shimmin: Yes. Type I is ++-- and Type II is either -+-- or ++++.

Herman: What about --++ and other IMViC types?

Shimmin: These are not data that I did myself. These are data that were collected in our region in 1968. IMViC Type --++ was 0. This is kelp process waste that was not mixed with domestic waste, but had the addition of sea-gull droppings. When the processor brings the kelp out of the bay, there are all kinds of fish and other materials caught in it. So, when it is being chopped up and transferred.....

Herman: Did you say Type --++ was negative?

Shimmin: Yes.

Herman: What is represented then when you say *Klebsiella-Enterobacter?* Are you essentially taling about *Enterobacter* and not *Klebsiella?*

Shimmin: No. I am talking about two different things. The first section of data that I talked about from the San Francisco Bay is not IMViC data; they are Enterotube data. That is when I was talking about the *Klebsiella-Enterobacter* group. The second set of data were IMViC data from the San Diego kelp processing.

Vasconcelos: Which Enterotube are you talking about? Was it the new improved Enterotube with the ornithine?

Shimmin: It does not have ornithine.

Vasconcelos: They now have included ornithine. They solved the *Klebsiella* problem; because, I understand the system was not working too well for *Klebsiella* identification.

Shimmin: Right, that is why I called it *Klebsiella-Enterobacter*.

Resi: Do you ever perform the biochemicals to check out what you got with these Enterotubes?

Shimmin: We did not do it on this particular study because we only gathered the data last week. But we have checked the Enterotube results we have gotten with *Salmonella* isolations and they have always confirmed exactly what we were getting in the data in the individual media.

Knittel: That Enterotube is designed for *Salmonella* and related work; it is 100 percent in the isolation. When one starts digging into some of the other *Enterobacteraceae,* one is going to be in trouble because the percentage is not that high; I think it is something like 85 percent positive.

Bauer: Excuse me, but I hope we do not get too involved right at the moment in the details of what we are identifying. We are still trying to come to grips with the problem: Should we apply a bacteriological standard to the pulp mill wastes?

Herman: We are not talking about pulp mill wastes.

Bauer: Industrial wastes, more specifically, one that has domestic sewage excluded. Are there any questions for Miss Shimmin?

Dr. Leonard Guarraia: Are all these out of sea water, Miss Shimmin?

Shimmin: These are effluents. There is sea water in the kelp effluent, but the cooling water and all the source water in industries along San Francisco Bay are primarily fresh water, I would imagine, because of the location of the plants.

Riordan: Could you relate this to the elevated temperature fecal coliform test?

Knittel: That is a misnomer. We had better put that to rest right now. That elevated temperature coliform idea is the biggest bunch of garbage they have come up with.

Riordan: But this is what they are saying.

Knittel: Just recently, I asked a representative of the Council, "What is your definition of an elevated temperature coliform?" He said that it is anything that grows on the FC membrane at 44.5°C. Well, there is a lot of different bacteria that grows on there.

Questioner: Whether it is blue or not?

Knittel: Yes, whether it is blue or not. I said, "Now, what about *E coli*?" He said, "That is a different ball park." He began to back off on this question. The only thing the National Council is really going to believe at this point in time is *E coli*.

Bauer: May I ask Miss Shimmin a couple of questions? On these particular wastes we are dealing with, you mentioned that with the kelp processors, there was evidence of rapid multiplication of bacteria in the processing of kelp.

Shimmin: That was a hypothesis, because of the kelp processing itself.

Bauer: Yes. Is there an indication of that type of thing occurring in sugar processing, i.e. considerable regrowth through the process water?

Shimmin: We have not checked it that far. There is a great reluctance on the part of the industry to let us come in and sample. Therefore, we were only able to sample at selected effluents.

Bauer: May I press the point a little bit further then? You said that, with the kelp processing wastes, there was a good chance that fecal contamination from sea gulls was mixed with the raw product as it entered the plant. What would be your judgment with reference to sugar cane?

Shimmin: Well, sugar cane, as it comes from Hawaii, is rather dirty; it has been rinsed but it certainly is not sterile, so I would imagine since in Hawaii they do use some sewage effluents to irrigate the cane fields, that there is a possibility of contamination.

Bauer: They also have a large rat population. Isn't that somewhat of a problem?

Shimmin: Yes.

Geldreich: I have a question, when trying to interpret this last page. Are these individual numbers, where you have "total and fecal coliforms per 100 ml?"

Shimmin: These are averages.

Geldreich: What is bothering me is that you show total coliforms less than fecal coliforms, after talking about lactose fermenters.

Shimmin: Yes, but this is according to the appearance on M-FC plates when the value is greater than 60,000; these are blue colonies that appear.

Geldreich: You mean to tell me then, that you found lactose fermenters, which are blue colonies on the M-FC plates, that were greater in number than 60,000 and then, only 23,000 which will ferment lactose at 35°C? This is very difficult to reconcile because we are talking about lactose fermenters. Your lactose fermenters at 35°C are more numerous than they are at 44.5°C.

Shimmin: That is true and that is why I made the remark when I was reporting the data, i.e. that this was highly unusual. I believe that those were all fecal coliform colonies that are appearing as blue colonies.

Geldreich: What were the densities of organisms on these total coliforms you used on the MF? Are we talking about numbers like 23 on the MF or 80?

Shimmin: We are talking about statistically significant values.

Geldreich: What was that?

Shimmin: On totals, that would be twenty-three colonies per membrane.

Geldreich: What was your background count on these membranes?

Did you have a problem? I wonder if we have an overloaded membrane problem.

Shimmin: No.

Geldreich: You say that you do not have one. That is why I cannot reconcile something that will not ferment in the same magnitude of population at 35°C but it does at 44.5°C.

Guarraia: Did you try this with different lots of media?

Shimmin: No.

Vasconcelos: You say this was an average. Is that correct? One could get some distortion if one has a high point, a mean in other words. How much data....

Shimmin: I lifted the data myself and this is what I would consider statistically significant.

Vasconcelos: Is it not skewed?

Shimmin: No.

Another question: What you are asking is, "Is it an arithmetic or geometric mean?"

Vasconcelos: Right. When you say average, right away.....

Shimmin: It is an arithmetic average, but the range of counts was not a great enough range that one would need to use the geometric mean.

Vasconcelos: It looks, you know, a little.....

Shimmin: It looks very strange, but that is my point. These things are appearing on the M-FC plate.

Bauer: Have you experienced this frequently? Is this the only time?

Shimmin: This is when we first started running industrial effluents. We did not experience it in any of the sea water or sewage types.

Vasconcelos: You said the San Joaquin River.

Shimmin: This is the San Joaquin River, close to the intake, and I have a feeling that there are other discharges from the pulp mill coming down. I don't think you are looking at anything too much different than from the pulp mill itself.

Bauer: I would like to cut this particular point off. Perhaps we can bring it up again. Tomorrow I would like to talk about methodology a little more. I am hoping now that we can continue to focus our attention on the question whether or not bacteriological criteria or effluent-limiting criteria should be applied to industrial wastes.

Vasconcelos: Will the pulp mill companies agree on a bacteriological standard?

Bauer: No, they will not. That is the whole problem. They say that we are way out of line to even consider suggesting one.

Vasconcelos: I am talking about an *Escherichia coli* standard.

Bauer: They would agree to an *Escherichia coli* but not fecal coliform.

Geldreich: If the difference is two million versus twenty million; so what, we still have a problem. Taking your data, Dick, where you might have EC as you partition it off, maybe two million on some of them and your total fecal coliform or your *Klebsiella* counts were even higher, the magnitude is tremendous. We have to achieve reduction. This is a smoke screen the companies are throwing out.

They tried three different ways. They tried to get EPA to get around using the word "fecal" because that is a nasty word. They won't look for *Salmonella* which I have asked them repeatedly to do. They do not want to carry out this reduction, which is the problem, and they have attacked the methodology in terms of an MF and MPN problem. Now they are going on the idea of trying to throw a smoke screen on what we find in our methods, like *Klebsiella* versus other coliforms, not realizing of course, that *Klebsiella*, as we find, are found in some human intestinal tracts and other warm blooded animals. It is a series of probes that they are trying to put out to cast confusion over the real crux of the problem: Have they got the nutrients down?

Riordan: I think that is the thing we have got to do. Sit down and decide whether we have a method. I don't think one can obtain the desired result on the basis of 30 mg/l. There will be some nutrient reduction, but we do not have the same experience with pulp and paper wastes that one has with municipal wastes. Five-day BOD with paper wastes has a ratio, I believe, of about 20 percent ultimately, rather than the 67 percent that we have in municipal wastes. This means that even with 30 mg/l one is going to have the nutrient there.

Geldreich: To start with there are more sugars there.

Riordan: One may not be able to think even of 30 mg/l as being adequate nutrient reduction.

Geldreich: I think that number is the workable thing that we have to look at really; it is between 14 and 30 mg, from our own experiences. I would like to check it out more thoroughly.

I would like to let Dr. Martin Knittel present his data. Then perhaps we can get down to some serious discussion about the points raised by Mr. Stein this morning.

Review of Research Regarding Coliforms in Pulp and Paper Mill Wastes

Dr. Martin Knittel

I would like to give a progress report on research we have conducted during the last eight months, on the presence of *Klebsiella pneumoniae* in pulp and paper mill wastes.

We are interested in the ranges of *Klebsiella pneumoniae* in various pulp mill and fiberboard wastes and in the local streams. From this data we would develop a research plan to meet the objectives of the program. Our isolation technique is very easy because of the large numbers of *Klebsiella pneumoniae* in pulp mill effluents. We have found that close to 100 percent of the total coliforms appearing on the M-Endo agar can be identified as *Klebsiella pneumoniae*. We picked these colonies and innoculated them into lactose broth and those that were positive for gas in forty-eight hours were transferred into the IMVic media and ornithine decarboxylation medium, checked for motility, and typed using pooled sera. A representative number of these was sent to NCDC for confirmation.

Some brief observations on the *Klebsiella pneumoniae* that we have isolated: They are usually late lactose fermenters, delayed forty-eight hours. For some *Klebsiella* there is a temperature dependence on gas production in lactose. Some are not positive at 35°, but will produce gas in lactose broth when incubated for an additionalfour or five hours at room temperature. Not all of them are encapsulated so not all of them are typeable. They make up between 90 and 100 percent of the total coliform colonies found in pulp mill effluents. We have been able to identify *Klebsiella pneumoniae* following any of the criteria for identification. Because there are a number of classification techniques, one of the problems was to determine if the isolates were either *Klebsiella pneumoniae* or some other *Klebsiella*.

Let us run through these slides. (A series of slides demonstrated *Klebsiella* colonies and growth curves).

These are cultures that we received from the American Type Culture Collection that we wanted to use as comparative organisms. We plated them out to get some idea of colony morphology.

Geldreich: Notice that on overloading the membrane bigger colonies occur toward the edges while the center ones are sometimes quite tiny.

Knittel: Right, we made no attempt to count these colonies. We merely wanted to see what kind of reaction would result on M-Endo and LES agars. The next slide is more typical of a pulp mill effluent. The mucoid colonies show a variation in metallic sheen production, a rather soupy type of colony. This is more typical of pure cultures than have been carried in the laboratory for some time.

Geldreich: Is the alcohol in that particular LES media? It would keep down the confluency.

Knittel: Yes. This is a highly mucoid organism, difficult to count on the plates because it spreads very rapidly. Let us go to some of the data we have collected (Appendix E). On four successive days we sampled the mill for total-and fecal-coliform bacteria determination (Figure I). I do not have the fecal coliform data prepared, but they would present a similar profile with lower numbers. The intake water had low densities. As we got into the primary settling ponds, the numbers came up. There were day-to-day fluctuations in concentrations. When we looked at the secondary pond effluents, the numbers of coliforms were higher showing that regrowth was occurring during treatment.

The next slide (Figure 2) shows the comparison between the number of total coliforms and percentages of *Klebsiella* in primary and secondary influent-effluents. There is a large number of coliform organisms coming into this primary, indicating that they are coming out of the mill some place. Somewhere within the mill is the source of inoculum, in this waste, which increases in numbers during treatment. There is a slight but reproducible decrease owing to settling going through the primary settling phase.

As the waste enters secondary treatment, which is merely an aerated lagoon, there is an increase. There are more total coliform bacteria than there are *Klebsiella*. The higher numbers may represent the effect of pH adjustment as the effluent goes in the secondary treatment system. There is a long travel period from the primary to secondary. By the time the effluent comes out of the secondary aeration, there is approximately a two-orders-of-magnitude increase in numbers, most of which are *Klebsiella*.

Speaker from the floor: Is this the sulfite mill?

Knittel: Yes, this is the Crown-Zellerbach (Lebanon), one of the easier mills to sample because we have money invested there. We have trouble getting into the other places. I think if we could sample other mills we could show some kind of relationship. We found it in kraft mills and one other mill that we have sampled. There is a presentation, in figure 3 of the data on a grab sample of secondary influent, treated in various ways to determine whether regrowth of these *Klebsiella* and fecal coliforms could occur at the treatment temperatures the mill was using. We took a portion of the sample, incubated it, and called these total coliforms the indigenous coliform population. We compared this level to the coliform increase in the waste itself; we did have an increase. We sterilized the sample either by autoclaving it or by filtering it through 0.45μ membranes. We inoculated the autoclaved or filter-sterilized wastes with an EC positive, Type I, *E coli* or a *Klebsiella pneumoniae* which we had isolated from their system. We incubated it at 20°C, on a shaker in a waterbath. We got good growth as one would with any standard nutrient medium. We had a discrepancy with the filter-sterilized waste: we did not have much growth or die-off. This result may be explained on the basis that some of the nutrients that are available for growth have been removed by filter sterilization. The particular material may act to adsorb the nutrients onto the surface so that essentially they have been removed.

Speaker from the floor: You can always break them down in the autoclave, too.

Knittel: This is true. We may have solubilized the nutrients. These results did convince us that we, when sampling the various waste streams in and out of the various treatment facilities, were seeing an overall increase in coliforms and *Klebsiella*.

Geldreich: Did you try this with the *Salmonella?*

Knittel: No, we have not.

Geldreich: We have done similar experiments, and the *Salmonella* take off just like that.

Herman: We compared maximum growth rates of *E coli* and CDC-confirmed Type 33 *Klebsiella pneumoniae* at 15°C and they followed the same pattern. The *Klebsiella* grew faster than *E coli*.

Knittel: These observations probably come back to what we have been saying: Growth is really a function of nutrient availability. If these coliforms can grow in the plant, and if the plant itself is contributing nutrients, *Klebsiella pneumoniae* can grow. Then *Salmonella*, *Shigella*, or some of the other *Klebsiella* can also grow. This problem is not so much that the wastewater represents immediate fecal contamination, but that growth of the coliforms does occur. If these wastes can grow coliform, *Salmonella* or other pathogens may also grow. This increases the chances of water-borne infections. On the other hand, these large numbers of coliforms may mask coliforms from other sources that are more of a health hazard.

As a result of the Corvallis meeting, and of the statements that were made to the effect that *Klebsiella* could be found anywhere in the uncivilized environment, we went to an experimental forest, on the campus, which has limited access. We took samples of vegetation, pine needles, leaves from trees and bushes, leaves on the ground, soil, and water from a small stream. We found no total coliforms, fecal coliforms, or fecal streptococci (Figure 4). We did find some total coliforms in the water samples after forty-eight hours incubation, none of which were lactose positive. We did not carry them further but we hope to follow this up at a later time.

The representatives of the pulp and paper industry at the Corvallis meeting contended that they were willing to go along with *E coli* and they kept bringing up the "elevated temperature coliform" theory. They denoted "elevated temperature coliforms" as those bacteria that are found on the fecal coliform membranes, including typical and atypical colonies. I find this term very confusing and suggest that EPA microbiologists develop a statement to rid the future literature of this term and to define more precicely the term fecal coliform.

We investigated the effect of temperature on the growth of fecal coliforms at elevated incubation temperatures. We shall proceed very rapidly through these slides. This is an *E coli* (National Center for Communicable Disease, Atlanta, Georgia) plated at 44.5°C. It is a typical blue colony, easily identifiable as a fecal coliform. Next is the same organism incubated at 45°C. There is not much difference. The counts go down a little, but they will survive at 45°C. Next at 45.5°C the colonies are larger.

The next slide shows a *Klebsiella pneumoniae* culture from NCDC plated on M-FC medium at 44.5°C. This is the reason the pulp and paper people have been after this coliform problem. These organisms are the ones they are calling "elevated temperature coliforms." They are lactose-fermenting, thermal-stable *Klebsiella pneumoniae* that will look just like *E coli* at 44.5°C. They do grow at 45°C. At 45.5°C they do not grow. This colony appearance is not true of all *Klebsiella*. The culture from the American Type Culture Collection that I showed before were plated in a similar manner and incubated at those same three temperatures. They do not survive. We had no colonies appearing on the medium.

Next, we see the fecal coliforms from a mill effluent. This is the appearance of the colonies when the plates are incubated at 44.5°C. There are some dark blue colonies typical of fecal coliforms intermixed with some atypical colonies of various colors from white to pink and those with blue-centers. The next slide is a close-up one to emphasize the high numbers of atypical coliforms that crowd the membrane and obscure the actual numbers of fecal coliforms.

The next MF was incubated at 45°C; the background growth has been decreased and the colonies are countable. The problem is to demonstrate the presence of fecal coliform colonies intermixed with the atypical smaller colonies. A close-up of the previous plate shows that the suppression of the atypical colonies by a 0.5°C temperature increase makes the dark blue typical colonies more distinct. On the next plate incubated at 45.5°C, the number of atypical fecal coliform colonies has been suppressed further. When light blue or blue-centered atypical colonies are examined in culture they are found to be slow lactose-fermenting, thermal-resistant *Klebsiella pneumoniae*. The large, dark blue colonies isolated are about 100 percent *E coli* Type I and follow all the criteria set forth for typical fecal coliforms. At 45°C and

and 45.5°C one notices that the number of atypical colonies has been suppressed, and it is easier to define the fecal coliforms. The next close-up of the previous membrane shows atypical white, blue, blue-centered, and cream-colored colonies that are not *E coli*, when we compare them with typical dark blue, somewhat irregular *E coli* Type I colonies which gave the typical ++-- IMViC reaction.

The next membrane is more typical of the mill effluent, a very crowded membrane. We can pick atypical colonies without contamination. If we use a higher dilution we do not have a countable plate, just one or two blue colonies.

Geldreich: That is an overloaded membrane, though.

Knittel: If we go to the next highest dilution, we lose the *E coli*.

Geldreich: What is the next highest, five-or ten-fold difference?

Knittel: One-tenth, a decimal dilution. For example, this would be 10^{-1} and the next one 10^{-2}. The next membrane was incubated at 45°C. We immediately have eliminated many of the interferring atypical background organisms in the same sample dilution. The next slide is a close-up of the previous plate. We suppressed the growth of the slow lactose-fermenting *Klebsiella*, and exposed the typical type I *E coli*.

The size of the blue colonies, the fecal coliforms, is larger when the membranes are incubated at 45.5°C. Temperatures above 45.0°C inhibit cell division of *E coli*; however, cell growth continues and the cells become filamentous.

This is an interesting organism represented on the next slide. Of course, we are not going to count this one as a coliform at all because of its green color on this media. We have picked several of these colonies and performed several biochemical tests on them. They turn out to be *Enterobacter hafnia*. A close-up will reveal colonies with a blue center. They are slow lactose fermenters and will turn blue after longer incubation. At 45°C this organism still survives very well. At 45.5°C we have definitely lost some of those atypical colonies but the larger *E hafnia* colonies survive.

We took an average of several runs at these incubation temperatures on three different effluents. We plotted the numbers of fecal coliforms that appear versus the temperature of incubation (Figure 5). There really is not a lot of difference between the numbers that survive at 44.5°C and 45°C. However, with the increase of the incubation temperature to 45.5°C, only about 50 percent of the fecal coliform population survives.

The first 2 columns in Figure 6 represent a comparison of the two types of colonies appearing on membranes incubated at 44.5°C. We picked representative numbers of colonies and recorded the results from a set of biochemical and cultural tests. From the wastewater examined 89 percent of the dark blue colonies tested were EC positive. Further in this table we reported the typical and atypical colonies that appeared at the three incubation temperatures and compared those two types of colonies on a variety of media. The dark blue colonies gave the classical reactions for *E coli*. The light blue colonies were a mixture of *Klebsiella* and other *Enterobacter* species, usually *hafnia*, once or twice *liquifaciens*. The same picture remains at 45°C. At 45.5°C the fecal coliform count went down, but those that did appear were definitely *E coli*. The background colonies were *Pseudomonas* of various kinds.

We have also attempted on a limited basis to use the enrichment technique for *Salmonella* but have not been successful. On every occasion that we have tried to enrich for *Salmonella* we have been very successful in isolating *Pseudomonas aeruginosa* from the same wastes.

Guarraria: Have you ever tried to use internal control for seeding?

Knittel: No, we have not. I would like to conclude my remarks with what I consider to be two important recommedations:

1. In any survey a confirmation procedure should be followed for the coliform bacteria that appear on either the M-Endo or M-FC media. For example, since pulp and paper industries personnel have stated that *E coli* type I is their main concern, confirmation of colonies from these industrial wastes is particularly important. This information will provide a firmer foundation for legal action.

EPA needs a central bacteriology laboratory devoted to classification and confirmation not only of members of the coliform group but also of other problem-causing microorganisms. We have been depending upon the good nature of NCCD to confirm enteric pathogens. We must develop our own expertise.

2. EPA needs to develop a document that clearly states the correct classification of the members of the family *Enterobacteriaceae*. (Some microbiologists are still using the outdated taxonomic keys of Bergey's Manual). EPA must refute the term "elevated temperature coliform" that is being proposed by the industries. This term is confusing and does not have a sound foundation.

Gallagher: Let me ask you this one question. You indicated that at the symposium in Corvallis about two months ago, the industry said it would be amenable to accepting *E coli*. Did the representatives say that?

Knittel: I do not think that they said that directly, but that was the way they were pushing it; they put great emphasis on the presence of *E coli* type I as being significant.

Bauer: There were certain members of industry, such as Matthew Gould of Georgia-Pacific, who indicated that, in trying to clarify the significance of the elevated temperature tests. They believe *E coli* is a lot more acceptable than fecal coliforms.

Bauer: I think the representatives would accept the presence of *E coli* as evidence of fecal contamination, with all that that implies.

Geldreich: I am very much impressed with what you're doing here on temperature. I should like to give you a little background on elevated temperature tests. When we began to work on the elevated temperature concept and looked through the literature, we found elevated temperature tests recorded back as far as when Eichmann was playing with the principle using glucose broth. Through the history of elevated temperature tests there have been many choices made as to what temperature to use. We finally settled on lactose broth and we started incubating at 45.5°C. We were much concerned that we were very close to the upper limit where we would get a sudden and very sharp kill-off if the water-bath deviated very much. We were also aware of the World Health Standards which were set at 44.5°C. We began a series of experiments with the environmental waters in our area at 44.5°C, 45.0°C, and 45.5°C. We found that when we reached 45.7°C, we had just about wiped out everything. If we found a colony growing at 46°C, it was one of these oddballs, a huge colony.

Therefore, we had a real problem to face in the laboratories that were going to do this test. We know that many of the laboratories did not have a reliable waterbath (a temperature deviation ±0.5 tenths or less). We felt that if we put out this procedure at 45.5°C we would run a very good risk of having people come up with negative answers because waterbaths would not be performing correctly. We set the incubation temperature of the test at 44.5°C to be sure that waterbaths, at that time, would be able to cope with this test (it is really very sensitive to temperature).

The World Health Organization is using, at this time, 44.5°C in its standards. I had the opportunity to talk with the water laboratory directors of twelve European countries last month in Belgium on another assignment and discovered they are all using 44.5°C. They may call it an *E coli* test; it is actually the MPN version with a confirmation very much like we perform. They are using a different medium, but I am sure if we made measurements under similar conditions, we would get identical answers. We have done some parallel tests with MacConkey broth and other media. We are talking about the same numbers and the same population worldwide.

In the light of your findings, it might be desirable to elevate the temperature of the test to 45.0°C now that we have more laboratories using better waterbaths. I do not want to go to 45.5°C because I am afraid of the results. We have to recognize a trade-off here.

Knittel: You can see from that curve that there is a plateau between 44.5°C and 45.0°C. The results are going to depend a lot upon the kind of population present other than *E coli*.

Geldreich: You made one interesting observation, that if one would incubate the membrane filter for the total plate count at 35°C for the normal time, but then set the plates out at room temperature for a few hours, one would see more sheen colonies which one would then pick. Am I reading this right?

Knittel: I was not referring to the colonies that appear on the total coliform plate, but to the isolates from the colonies growing in lactose broth.

Geldreich: This is a very critical thing when we are looking at this whole problem. For instance, on an elevated temperature test we must place the membranes in the incubator within thirty minutes and then we must take them out and get them counted within thirty minutes. On this particular test I have had many people tell me they leave the plates setting on a work bench for several hours--while perhaps they are having lunch, etc.--and then they count them.

Knittel: They might as well throw them away. They do not have typical blue-centered colonies. They immediately are released from the inhibition of temperature and they're going to grow very rapidly. Within an hour and a half to two hours they will change.

Geldreich: This also occurs on a total coliform test. The counts become excessively high because you suddenly get the slow lactose fermenters occurring in such populations. Instead of having 20 or 30 coliform colonies when you count, you might have 100. I do not think this is well understood.

Knittel: I do not believe people are following the procedures that strictly. I believe that there has to be precise quality control of media use, sterilization, and techniques to reproduce results within defined limits.

Geldreich: Both tests involve a transient reaction. With the total coliform test actually the *Enterobacter aerogenes* and *Klebsiella* will grow very fast. I was wondering whether those plates that you made had been out on the work bench for a while.

Knittel: Yes, by the time they were photographed, they had been out a while.

Geldreich: I have had this problem. I was going to photograph some colonies like this, and they had lost their sheen. They have completely consumed all the lactose. They have now started to attack the aldehyde, and then the color turns gray. They were originally sheen colonies and coliforms.

Brezenski: On the question of 45.0°C as an incubation temperature, this is also being perpetuated by FDA right now. At the last shellfish meeting in Washington, this was one proposal which came up regarding 44.5°C and 45°C incubation.

Geldreich: I do not think that you are going to find much difference in coliform IMViC patterns between 44.5°C and 45°C. If the higher incubation temperature will clear up the background of confusing organisms, I am all for it. I have looked at this very carefully, and I do not think that changing 0.5°C is going to upset anything we have done in the past or will do in the future. If it will help in paper mill wastes, I would be all for just making a standard across-the-board change to 45.0°C, in the next edition of Standard Methods. We had originally set the temperature to be compatible with World Health Standards and to recognize the problem with incubators at the time. We have reliable equipment now, and perhaps we ought to raise the temperature to 45.0°C.

Brezenski: I think that is a good point.

Knittel: We use Blue M Magniwhirl waterbaths, cover the tops, keep an extended scale thermometer on them, and check them two or three times a day. As well as I could judge, these plates were within 0.2°C variation. The temperature ranges from 44.9 to 45.1°C, but it averages out that the length of time at 45°C is greater than at the two extremes provided one keeps the waterbaths covered.

Geldreich: The important point to remember also is that this is still a fecal coliform test and not and *E coli* test. We are going to find *Klebsiella pneumoniae* even at 45°C and it is important. Also, the members of the fecal coliform populations may differ even within one individual. For a period of time a fecal coliform with one IMViC pattern may predominate, and then, three or six months later the population may shift into another pattern. That those organisms are all elevated temperature positives, is the important thing.

Bauer: I would like to bring this discussion of methodology to a close here if I may. If you are in agreement, let us start the discussion of the question at hand: "Is a bacteriological limitation necessary in industrial wastes where municipal waste is excluded?"

Brezenski: This is not a separate question. This question is tied very closely to the one we are talking about. This is methodology, also definition: what organisms we are talking about. Apparently, there is not complete agreement on the significance of some of these intermediate types or of *Klebsiella*. I do not think we can make a decision on one problem without considering the other. I think this interpretation is important.

Bauer: Let me be more specific. Do you feel that fecal coliforms at 44.5°C are a relevant parameter to apply to industrial wastes?

Brezenski: I think that is the more pertinent question.

Howard Davis: What do you mean by fecal coliform?

A speaker from the floor: Any coliform that will produce gas at 44.5°C.

Bauer: We are talking about how one definition fits on top of another. Coliforms that will grow and ferment gas at 44.5°C regardless of IMViC type, are good fecal coliforms.

Geldreich: *Klebsiella* is a coliform, a lactose fermenter. Sometimes, some of us forget this.

Brezenski: I agree.

Gallagher: Maybe you can be a little more specific. Some of the material that has been prepared by the NFIC, to go to the regions as guidelines for the permit program, has "organisms isolated in the fecal coliform test and associated with pathogens shall not exceed 1000/100." Is that a defensible statement? Do the processed wastes have sanitary significance? Secondly, if so, are the fecal coliforms or the organisms isolated in fecal coliform tests valid indicators of health hazards in these wastes?

Geldreich: This is a loaded statement.

Gallagher: I know that it is.

Baker: Can we take them one at a time then? Are we to be concerned about the high bacteria counts in industrial wastes, per se; do they have potential pathogenicity associated with them? If we can agree to that, then what kind of a test do we apply to it to determine that point? Alright, anyone willing to give his opinion?

Geldreich: First of all, if we are talking about fecal coliforms, regardless of whether there are 3,000 or 20,000, we are talking about fecal pollution. This is what we are trying to measure. We are trying to measure fecal hazard where there is no pathogen test that will guarantee that one can recover all possible pathogens that will be present.

Now as far as *Salmonella* is concerned, it is great when you get an answer. However, when one does not get an answer, first of all, it does not mean *Salmonella* are not there. I had a project, in the Northwest Laboratory, in which we had been looking at serotype recoveries in many different kinds of media and determinations for *Salmonella*. It is most frustrating because you cannot be sure they are not there when you get a negative result. Secondly, *Salmonella* is only one pathogen we ought to be concerned about. We have a man here interested in virology, and we have enteroviruses. I am also interested in enteropathogenic *E coli*, which is a fecal coliform. There are also *Shigellae* and *Leptospira*, if we were to talk about feedlots. The one thing that we can correlate best with this concern for pathogen hazards is the use of some procedure that will measure fecal pollution. The only thing we have in bacteriology is a fecal coliform test that correlates between 90 and 95 percent of the time with this type of pollution.

Brezenski: Let me stop you right here. The question that was put to me by the Council at that point was, "Yes, you can show data. Mr. Geldreich has shown us in the literature that one arrives at 95 percent correlation when one is working with sewage and human excreta. However, this fecal coliform test was not developed for use with industrial waste, and one will not come up to a 95 percent value in such wastes."

Berg: The problem here is that one is talking about two different things. One is talking about organisms as indicators on the one hand, but that is not what the issue really is here. The question here is: What kind of organisms....(interrupted, mixed talking).

Stein: As many of you know, I am kind of an amateur scientist and I am very sympathetic to what you are doing here. Let me tell you how difficult this is. We are going to have to determine whether there is a pathogenic significance (and we might want to stretch pathogens if you do not think we cover viruses) in the pulp and paper mills and sugar beet wastes. If there is a pathogenic significance, then there has to be a measurable limit, and this is probably going to be a coliform test. The point is: Are we going to say a high fecal coliform count in these wastes is a hazard to health? This is the hurdle we have to get over. I think we can back you all with the kind of test you set.

Geldreich: What you really want is definite, positive evidence of the pathogens present in an effluent as some of the states do....

Stein: No sir, we do not.

Gallagher: We want to have some evidence that there is a hazard associated with process wastes from the industries.

Lyons: If you cannot establish that, everything else is a lot of good scientific significance, but in the enforcement field we are trying to build a house without a foundation, and we have to have that foundation.

Geldreich: Now, you have fecal coliforms present, indicating a fecal contamination. What you want is to correlate it with potential pathogen occurrences if possible.

Gallagher: We would like to have what evidence is available.

Stein: Let me read this. (It is a very good and accurate explanation of the industry's position). "Pulp and paper industry research has shown that *Klebsiella* is often found in process waste waters that are completely isolated from the domestic wastes; it has no sanitary (fecal) significance." The statement is credited to Mr. Blosser. To me it is unacceptable. A waste does not necessarily have to be associated with sewage or packing house waste to have significance. Let us assume that we have an industrial waste like sugar beet or pulp and paper effluents where the process wastewater is completely isolated from domestic wastes. Do we have to control these waters in any way to keep the fecal count down? What significance do they have for man?

Geldreich: It is to tell them something they do not want to realize: There is so much nutrient present that those organisms are multiplying out of proportion. There are two things we are getting out of this indicator concept: One, there is fecal pollution there, and two, industries are magnifying the hazard because the nutrients are there.

Stein: Right. You made that point very clear. If we can convince the industries to keep the nutrients down, the industry will buy that and spend a lot more money than to keep pathogens down.

Geldreich: There is a method, the fecal coliform, to measure whether or not they are reducing the nutrients, because coliform counts are going to go down after adequate treatment.

Stein: If the right policy is to keep the nutrients down and we have some measure for nutrient reduction, we can put in a tight control. If, by controlling the nutrients, we are automatically going to get the bacterial and virus count down, I think we can push for a much harder restriction. If we put the control on the nutrients, and if we can get the industry to accept it, we are on firmer ground, particularly if we get into the area of arguing about the effects of the microorganisms.

Geldreich: There is one thing, one cannot rely on the chemistry test alone to be sure that nutrients are down. One has to have a back-up test in order to show that the coliforms are there in smaller numbers. We cannot be sure that the BOD or some other chemical measure is that good.

Stein: We are talking in terms of nutrients and we figure that the nutrients provide a medium for bacteria to grow. We just want the nutrient level down. Let me try to oversimplify: I think a company might get excited if I were to say they are pouring a test tube of bacteria in the water and contaminating the stream. But if I tell them they are dumping into the water some molasses which is relatively benign although it will provide a good medium for the bacteria to grow on, I can tell them to cut down on the molasses. If we say we want to keep these nutrients down and we are using this coliform level as a check, this may be a way of getting around first base.

Berg: May I raise a question here? I still think there is something very wrong with these arguments. Unless we can show that the *Klebsiella* are pathogenic and should be kept out for that reason, the question that is going to be asked in the situation where one is obviously getting amplification is: "How many organisms of fecal origin did you have originally since you now consider them such a problem?" Are the nutrients also bringing about the multiplication of pathogenic forms? If they are not, you are dead.

Geldreich and others: They are. They are.

Berg: I mean pathogenic forms.

Geldreich: Ralph Christienson has found *Salmonella* in paper mill wastes.

Berg: Then it is based upon the pathogens; what are you worrying about?

Geldreich: We are trying to establish, if we can, a policy from the standard procedures that we use. If we spend our time searching for pathogens all the time, we are really going to have some great problems.

Lyons: Are the nutrients you are talking about not soluble substrate levels or are you talking about this total group?

Geldreich: I am talking about a nitrogen and carbon source. I am vague because I do not know how else to put a handle on it.

Lyons: Because you could have it in either a solid or soluble form.

Knittel: Well, the nutrient has to be soluble and degradable, in usable form. A. W. Anderson, at Oregon State University, carried out some research related to slim growth control in pulp and paper mill wastes. This was a very sophisticated gas chromatographic analysis and it was beautifully done. He was able to point out that the six carbon sugars were very rapidly removed during the first portion of the treatment process. The five carbon sugars were left. They are a very good substrate for most of the *Enterobacteriaceae* including *E coli* and *Klebsiella*, so that we have really developed a selective medium for them. There is a tremendous amount of nutrients in these wastes. He calculated that five pounds of carbon sugars were going over the weir daily, based upon the flow. The number in tons was fantastic. A seven-day retention time is apparently not long enough to reduce them.

Geldreich: Other people have tried to measure nutrient levels with a chemical test. Lou Resi, Lois and Carl Shaddix, and others have tried to find some chemical test that will relate to this nutrient problem. We have not yet found one that is completely reliable. That is why I am cautious. Use the best chemical measurement whether it is

CCE, BOD, total carbon or another. However, we still need a bacterial test as a back-up in case that one system is not measuring all the nutrients that might be degradable. That is my only concern.

Stein: Assuming the complete separation of these industrial wastes from the domestic ones, we have always had doubts about whether we could relate the microorganisms present to a pathogenic hazard. We want to stress practical treatment in the permit program. As far as I can see, likely this is going to be the treatment that the industry is going to use between the years 1976 and 1981. At this stage, we may be able to get a big start on this program if we talk about keeping the nutrients to the minimum, with the exception that the five-day BOD test is, at best, a very rough operation. We can get more definitive results by having a bacteriological test. This may very well be the fecal coliform test. In one sense the fecal coliform test may be in a research stage for the job of relating it to the presence of a health hazard in these wastes. Still, we have to use this test as the only certain back-up that we have to be sure that we have controlled the nutrients coming from the plant. We may have to adjust the numbers if we use the test for this purpose. If the statements made here today are correct, treatment technology is available to reduce the nutrients in these wastes and also lower the coliforms. The correlation is pretty good and we will not have these coliforms in the effluents.

Geldreich: I think we have a two-pronged problem. One is to keep the nutrients and the bacteriological counts down, and the other is the problem of the downstream user. If the industry started out with poor source water, let us say 1,000 fecal coliforms, and they knock them down and still come out with 1,000 fecal coliforms/100 ml, we have some problems.

Stein: The point is, what we are doing here is not going to solve every problem. In the permit system we cannot hold the industry responsible for what they take in from a poor source water if that water comes from the same stream. We probably have a better line-up on holding them responsible if they take it from an underground water supply. For example, we could never hold an industry responsible for mercury in the water when they picked up the mercury in the intake water.

Geldreich: You are hoping that what goes into the system at least comes out no worse.

Stein: Yes, no worse than they take in. This regulatory permit program is what we are going to have to live with for the next decade unless there is a tremendous change in thinking.

Geldreich: If there are polluters upstream of an industry we can get a hook on them.

Stein: That is different. But if an industry takes in water with 1,000 coliforms/100 ml, they are going to be able to put out 1,000. We just hope the industry runs the mill right.

Bauer: In pulp mills some of the sources of large inputs of coliform organisms into the treatment system are the barker wastes, the white water from the paper mill, and so forth.

Knittel: You experience this tremendous proliferation of the organisms once they get into the biological treatment systems or in the primary clarifier.

Bauer: Industry has rather expensive water treatment systems for intake water at most of these mills.

Herman: They do not consider these costs?

Bauer: Yes, industry does, but it is forced to live with them.

Stein: It's a 'production' cost.

Herman: Why is not nutrient removal in waste a 'production' cost?

Stein: The answer is obvious. The industry went into that plant knowing what they had to pay for the raw materials. They consider water as a raw material. They consider cost of getting that raw material into shape a 'production' cost. However, once the industry gets the water in, they have a blind spot for spending a nickel to clean it up before they dump it into the receiving stream.

Gallagher: A proposal has been put forward to form the rationale for the permit system on the basis of reducing the nutrients and making the industries measure this reduction by some test, presently undefined, and to use the fecal coliform test as a monitoring device to be sure that fecal coliform values are being reduced to prevent the regrowth. That is the proposal. Is there any problem in using that kind of rationale in the permit program?

Davis: We seem to be talking about two things. One part of the problem is using the tests as a monitor. We are trying to institute a civil suit against a paper company and show damages. The other part of the problem we have been discussing is the sanitary significance. We are going for an injunction and maybe treatment later.

Gallagher: I think we all feel that there is sanitary significance involved here. The problem is a real logistical one in having to prove that in every case if we are going to get a permit. If one is going after a civil suit, one should have to prove that. But we are talking about hundreds of permits that have to be written, and about trying to keep the major logistical problem away from the Surveillance and Analysis Division, not having to prove it in every case. We are looking for a rationale to get the results and to put to the industry in a rational way.

Shimmin: I think concomitantly, if we are going to talk about nutrient removal and using fecal and total coliforms as indicators, we should have a statement in there about not allowing disinfection. If one is using total coliforms and fecal coliforms to indicate whether or not the nutrients have been removed, and the industries have added these dumps of chlorine, the indicators just might be temporarily wiped out.

Another speaker: Who is chlorinating? I do not think most of these industries are disinfecting.

Shimmin: My point is, if we do not have some other way of testing the amount of nutrients in there besides growing these organisms....

Stein: I know of no pulp and paper industry that chlorinates their waste, do you? Now, if you are going to open some shellfish beds, this is a specialized problem as I indicated to you before. If you get over 70 coliforms, you have the shellfish beds closed. If the pulp and paper plant or anyone else is putting in something that goes in the shellfish, you have a damage case, and you are going to have to work that out in special terms. I think your point is well taken. It might be possible to get your sample before you put in the chlorine for monitoring purposes.

Riordan: If you have a control on BOD, and you have a control on organisms, how is the industry going to escape? If they chlorinate, it is not going to bring the BOD down.

Speaker from the floor: You chemically oxidize the wastes with the chlorine.

Geldreich: I should like to forget about total coliforms. I do not think we are getting any additional information out of that test. Let us stick with the fecal coliform test. I think we are moving out of that area in the development of drinking water standards and in the development of the new water quality criteria. We are going to the fecal concept that we are phasing out of total so let us not continue to carry it on the books.

Gallagher: Do you think you can sell it to the shellfish people?

Geldreich: We are working on it. It is hard.

Stein: I agree with you, but you see what you are up against with shellfish. I have not been able to get the shellfish monitoring people to change. Here is the problem. We are going to be able to approach every paper mill with this concept except the paper mills upstream of shellfish areas.

Geldreich: In relation to the shellfish, let me give you something you may not have seen. This letter is dated April 24 to Mr. Kelly in our Office of Water Programs from Robert Schneider, Chief Biology Branch NFIC-D, who is chairman of a committee on shellfish testing. The point is that the committee is not going to total coliforms. That is rather interesting.

Gallagher: Yes, but the Food and Drug Administration is our problem.

Geldreich: My problem with them is that they call their test a test for *E coli*, rather than fecal coliforms. For shellfish we probably need another paragraph that zeros in on this specific problem in the permit policy.

Stein: We have that paragraph. With the shellfish we are going along with the total coliform parameter. The real test will be to see whether they are representing the shellfish concept. I do not think there are too many paper mills upstream of shellfish areas.

Speaker from the floor: What kind of tests, other than the fecal coliform test, would you suggest could be used to control nutrient levels? Total organic carbon?

Bauer: That was the chemical parameter that showed some correlation with bacteriological levels in the mills that I investigated.

Gallagher: The objection that the industries have to total organic carbon (TOC) has been "who owns the stock in Beckman and why do they want us to buy these $9,000 instruments?" That is the problem; they are not equipped for TOC work and they do not want to make that investment in instrumentation. They suggest they will live with the COD. There are a lot of problems there, too. Why do you not discuss what kinds of tests would be the best technically? We need a rationally defensible test for accomplishing this.

Geldreich: Looking at the data that we have obtained from field surveys over the years where the chemistry tests were run in parallel, we find difficulty in zeroing in on any one test like TOC or BOD. I cannot give you that answer right now.

Stein: One of the problems here is cost. Some of the larger companies will do these tests, but if you expect that of a canning plant, forget it. That plant does not even want to spend the money for labor costs to send an employee out to the end of the lagoon to take a sample and return. The possibility of really securing s sophisticated test into all these permits is practically nil. If you include as a requirement for this permit that these sophisticated tests be carried out, you are going to get a tremendous complaint, and my bosses and yours are going to sympathize with them.

Geldreich: The only thing that we can do then is to emphasize that the fecal coliform level input and output be identical and base it on that arrangement.

Stein: And have them check it?

Geldreich: No, I would not have them check it.

Stein: No, I would not give that up. This is perhaps one of the more useful things that we can do.

Lyons: May I invite comment from the S & A Division people as to what that would mean if they had to check all of these parameters, i.e. the fecal coliform and the TOC?

Bauer: I do not see the TOC as a big problem because it takes perhaps a 10 ml sample, which can be acid stabilized, placed in a bacteriological mailing tube, and tossed into the mail. It is a very quick analysis. I would not be at all adverse to all S & A Divisions being required to run TOC's.

Lyons: Is it a part of their regular monitoring?

Bauer: Yes.

Geldreich: That is the answer then. It would be the kind of information we would need. It would provide a ball park number, and we would back it up with a bacteriological test.

Lyons: Now, of course, the next question is that there is not sufficient data base on TOC. What number do you choose for the effluent? It will not be a monitoring tool until there is a number.

Stein: We agreed at least in the pulp and paper industry on that. The paper industry would be ready to use TOC instead of BOD or any other test if we could find the correlation.

Bauer: I do not think it would be too difficult to do it. Again, I relate back to the microfermeter work that was done at Crown-Zellerbach. You could perform TOC analysis using instrumentation, together with bacteriological tests on various kinds of mills, and see whether you can get a decent correlation.

Stein: The industries will not buy the principle that they have to put out the same water quality that they take in. Do they not add something in the plant?

Knittel: Can we go with a net increase type of statement?

Geldreich: That is the best way.

Knittel: A net increase that would be equal to the water quality standard for the water that they discharge into, or something like that.

Geldreich: The quality should not be any worse than what they took in.

Knittel: What I meant was a net increase through the plant. Is it entirely possible not to get any increase of fecal coliform through the plant? With adequate in-plant controls and best practicable technology?

Geldreich: I would certainly think it is possible.

Question from the floor: How is it possible to control within the mill?

Bauer: You can establish the kind of BOD level, TOC level, or whatever, that you consider acceptable and, which is indicative of best practicable treatment.

Remark from the floor: That has been done.

Question from the floor: Yes. What is the bacteriological level that would be associated with that?

Remark from the floor: That has not been done.

Lyons: Let us say that 30 mg/l might be the upper limit for substrate concentration.

Geldreich: It is somewhere between 14 and 30 mg/l.

Herman: I would consider 30 mg/l the maximum.

Lyons: This fits nicely except for the canning industry. The rationale in both the pulp and paper and the sugar industries is based generally upon 30 mg/l in the processes which are most used. We can ask them to go to bat with the TOC as a monitoring tool to develop a data base and with the fecal coliform as a back-up for monitoring. Do both of these tests have to be run by EPA?

Stein: For industries other than the pulp and paper industry, you are going to have to do the tests. I think the reason is obvious. The pulp and paper industry is a big business and they have a scientific staff. I do not think you are going to find that in the canning or in the sugar beet industry.

Herman: I have had seven years in canning, International Canners' Association. I know that when the canners wanted to treat, they did. From waste effluents between 1,600 and 2,000 BOD they produced a final effluent which routinely for over three years held at eight. They can do it.

Stein: Well, let us try them.

Speaker from the floor: That can be backed up. I do not think there is any problem. They will admit they just have not designed facilities.

Herman: They will recognize that they can do it.

Bauer: I am hesitant to give my complete blessing to having EPA perform all these fecal coliform tests, recognizing what it will take to get them done. The TOC tests can be punched out quickly on the instrument, but fecal coliform tests will require time.

Knittel: Fecal coliform samples cannot be preserved for later analysis.

Geldreich: How about state agencies?

Stein: That is what we may be able to do. The question is, who is responsible? This is the basis of the problem. I have been out in the field and looked at these canneries, big and small. These are seasonal operations, small plants. Some companies may cooperate with us and do the fecal coliform tests. The canning associations and some of the pulp and paper mills will. With other plants, we should probably indicate that we are going to monitor on a spot-check basis. As a regulatory device we do not have to do this with every plant.

Geldreich: I think once you have established a baseline to determine the TOC number, you will not have to do very much bacteriological work; it will diminish.

Stein: If we had the TOC we could go ahead. We do not have the baseline.

Gallagher: This would be set up as a monitoring device. When they feel the data base is sufficient, maybe two years from now, the permit program would amend the permits to incorporate the baseline which we are talking about. This is a way of getting at this whole problem of bacteriological distress.

Herman: We should have the authority to make this check when we choose.

Gallagher: That is taken for granted as part of the monitoring program.

Herman: That was not the case when I was with industry.

Stein: I would not give any industry a permit unless they would agree that any authorized representative can come in at any appropriate time and take a sample. Otherwise the firm does not receive the permit.

Berg: Are you talking about one TOC level for all industrial wastes?

Gallagher: No, we are establishing TOC as an indicator for all wastes but different levels for different kinds of wastes.

Herman: The National Canners Association was bucking some of these coliform tests; so was the National Council and the potato and sugar industries. You will have a united effort from all of these industries.

Gallagher: That is right. That is why we are having this meeting.

Stein: Do not feel bad about the Corvallis meeting. In my opinion it would have happened anyway. Every time we finish a meeting with an industry, their associations are going to get together. It was inevitable. It is very easy to talk with these industry representatives when you are discussing research. When you are talking about regulations, the situation is different.

We have this fecal coliform testing problem with all these industries. It appears to me that we are going to have a very hard time selling this permit policy on the basis of pathogen effect, viral effect, or health hazard, where we have a segregated waste. If we push the pathogen approach, the natural reaction would be to disinfect, and I am not so sure we want that because of the regrowth problem. I think the two alternatives may dovetail if we can push these guidelines to remove the nutrients. We may get the same results and be five to ten years ahead in cleaning up the water. It may be a blessing in disguise, to approach it the other way.

Gallagher: We are going to ask the regional programs, with their monitoring programs, to examine for pathogens and see if they can get a data base on them.

Geldreich: If you get positives, it is great, but when you get negative answers, you are just spinning wheels.

Another speaker: That is right - look at the Snake River Report.

Herman: In the 1966 meeting of the National Canners' Association many of these points were discussed. At least some people within the industry felt then that the Federal Water Pollution Control measures within three years would be what you are suggesting today. This would not be any shock to them.

Stein: I think that is right. When representatives of these industries go to a scientific meeting with you, they are constructing and testing a case that they throw at us when we meet with them. They have all our arguments before we put them out. The way they get those arguments is by going around with their scientific contacts. After all, where do we get our arguments, but from fellows like you. If they have tapped you fellows at the source, then when we have all the arguments, they have the rebuttal all lined up. I am not against free scientific information exchange with anyone, but please remember what they do with this information.

Bauer: Are we satisfied with the proposition that Mr. Gallagher has summarized? Are there any violent disagreements with that?

Knittel: Since we do not have a baseline, I am looking for areas of research to support this. Perhaps we need microbiological research to support these tests with regrowth studies and correlations of coliform content with nutrient level. Will this kind of information help the permit program?

Geldreich: You need authorization, and that has been on the books for a long time.

Stein: If you can pass up some of the bureacracy to get such research through, we shall back you.

Lyons: You will have the support, at least from our programs to highlight points with which we are involved, and problem areas that need more exploration and definition.

Knittel: We do not need a lot of money or a million dollars worth of equipment to do it. We do need people, but if we cannot find the people, we shall do it anyway. If we can get the support from the top level, we shall do as much as we can.

Stein: We can break through this impasse. We need your help. If you have a project that you think will go, just keep pushing it. When we have problems which are partly biological or microbiological that an engineer cannot solve, we will need an aquatic biologist or a microbiologist. When it comes down to the real expertise in your areas, you are going to have to make the judgments yourselves, because the engineers and ourselves are something like educated amateurs. By the time we had the second enforcement case years ago, we dragged Harold Clark (deceased) from the Taft Center because I recognized that we could not move without this information. I think that essentially the next law suits are going to be in the area where the people, in this room, who have the expertise are going to lead us. This is one of the areas where we are going to need some monitoring and sensitive tests. We have a pretty good record with

with the engineers and with the fish biologists. Now we are going to need some of the more sophisticated testing. We have to depend more and more upon the kind of information we have discussed here today. We shall have to work together in identifying the problems.

Let me say one last thing on this subject. No lawyer or engineer or aquatic biologist can tell you what you should do. You have to determine it yourself. I picked up more ideas by watching your presentations here today than I ever did from meeting with people who are not working in your particular field. You have to identify these research needs yourself and put them forward.

Gallagher: Let me say specifically, that if any of you people in Office of Research and Monitoring (ORM) has a particular project that could be of benefit to the permit program or in the enforcement program generally, let us know about that project. If you need the external support, and it is compatible with the enforcement program, we shall try to get you that support.

Shimmin: Could we hear again the statement that you are going to make about the coliforms?

Gallagher: We are not going to make any statement. We are proposing to summarize what has been said to you, and write up a statement based upon our understanding of the discussion. We will very quickly put it back out to you to see whether or not you think that is what you said. Then we shall make a recommendation to Headquarters.

Speaker from the floor: In other words, we shall all see that statement.

Gallagher: Well, I do not know whether you will all see the statement, but the summary will be sent out. We want to do this very quickly.

Stein: There will be no surprises in the statement.

Shimmin: Is this statement under the "Considerations for Permit Preparation" on coliforms going to be deleted?

Gallagher: Yes. This is one that was set up with the pulp and paper industry for the permit proposal to which they strenuously objected at the Corvallis meeting. I would think that this would be replaced, except for the last paragraph on receiving waters which are classified for shellfish harvesting.

Lyons: That was a sanitary engineer's poor attempt to set a number on bacteriological significance. We are looking for recommendations.

Stein: The real problem we have to face is that although we are going to have lawyers and engineers drafting permit and enforcement policy, we will have to work with these other professional groups, aquatic biologists, bacteriologists, and specialists in other fields. You, the microbiologists, are going to have to give us the formulation that we are going to sell. We are not going to have any lawyer or engineer or any specialist in Washington who is going to sell this policy and then face the rebuttal. We are dealing with your field, bacteriology, and the rebuttal is going to come from bacteriologists. We are never going to make it unless we get the formulation from you people.

Bauer: There are a few things we ought to discuss tomorrow -- additional research needs, problems with Standard Methods, and this type of thing. Another question we have talked around all day today is whether or not we should be concerned about the presence of *Klebsiella* in large numbers. Are they potentially pathogenic?

Stein: I can find another problem for you when you need it. I sensed it today, and it was proven by several other people in the room. We are going to have a lot of problems unless someone can get the methodology lined up so that it is uniform. I do not suppose you would want such tests as you are coming up with now in Standard Methods. I do not know whether you can split samples, or how you do it. I do not want to presume to tell you how to do your work. However, from what we have cut out for ourselves here today, the sooner you get at this problem of uniformity the better off you are going to be. Perhaps you can do this while you are all here, or perhaps you are going to set up committees. Even if you are all agreed now on what you are doing, when you go back to your various laboratories, carry out these tests, and start to get different results, you will wonder why the results are so disparate. Unless the microbiology checks out very closely, we are going to have another meeting like this six months from now to discuss these differences. Then we are going to have to get Mr. Gelreich and a committee of four others to go around from laboratory to laboratory to pull you together, and this is a wasteful activity. This is approximately six months off and unless you have coliform methods, you have problems.

Geldreich: I have some encouragement for you, Mr. Stein. Several of us in this room are on Standard Methods committees. I was on the committee for the last edition and we can handle getting it into the next edition of Standard Methods which we are now preparing.

Stein: In the interim this is the problem we are dealing with.

Geldreich: Right.

Stein: The point is, if you go away today and do not have your methods standardized, you will have problems. For example, one man may work for the next four months and come in with all those beautiful counts, but his results are a little different from those of another laboratory. So you decide he is not doing the tests exactly right. He is going to complain loudly because he has that tremendous investment in this data.

Geldreich: I do not think you will have that problem.

Stein: I hope not, but if not, this will be the first professional group not to have that problem.

Geldreich: We will discuss this more tomorrow, but I do not think we shall have any problem. We are closer to solutions than you think.

Gallagher: I should like to thank all of you for coming today because we are over a tough problem. I think that we have something that we can defend, and call upon you for, in our confrontations with industry -- our search is after truth with the industries.

Bauer: One of the questions we have been touching on at this session is whether or not *Klebsiella* is considered potentially pathogenic. Bill Stang asked Leonard Guarria to do a brief literature research for us in preparation for this meeting. To kick off the discussion of possible pathogenicity of *Klebsiella*, I should like him to review this literature for us.

Brief Literature Review of *Klebsiella* as Pathogens

Dr. Leonard Guarraia

Briefly, I think it is important to distinguish bacterial infection from disease. Infection merely seems that an organism is capable of living within a host. We are infected by many organisms - *E coli*, streptococci, staphlococci, and so forth. When the organism causes a disease, that is a pathogenic condition; it becomes important in terms of human health. *Klebsiella pneumoniae* infects approximately 30 percent of the human population. It has also been isolated as a saprophyte from soil. It probably is more significant as a human-related organism; at least this is my feeling from my search of the literature.

Klebsiella is a gram-negative asporogenous, non-motile rod which ferments lactose. That is the definition in Bergey's manual and in all the textbooks. As a disease organism it was first recognized as Friedlander's bacillus, and it was associated with upper respiratory distress, pneumonia. It causes approximately 2 percent of the total cases of bacterial pneumonia, and about 60 or 70 percent of the mortalities of all bacterial pneumonia are related to *Klebsiella pneumoniae*. This organism is resistant to many antibiotics and is so encapsulated, so slimy, that normal defense mechanisms of the host break down.

The *Klebsiella* have been associated with liver absesses, infant diarrhea and urinary tract infections. Many healthy people (10 to 20 percent) have been found to shed *Klebsiella pneumoniae* in their urine. The organisms are not in the kidneys, but occur on the way out. They have been associated with urinary tract infections and with oculitis, eye infection. *Klebsiella* have been cultured with increasing frequency in certain hospitals in human blood cultures. This is a rather serious finding by medical microbiologists.

Klebsiella have also been implicated in bovine infections, mastitis. Many healthy cows have antibodies to *Klebsiella* antigens in their blood. Many healthy animals (about 30 to 40 percent) have *Klebsiella pneumoniae* in their gut.

An a medical problem, *Klebsiella penumoniae* certainly does present some concern. As an indicator of pollution, it is a legitimate one. One cannot say, a priori, that because we have *Klebsiella pneumoniae* occurring as a high temperature organism, that it invalidates a test. That is an erroneous assumption. The elevated temperature test is still a very valid test in my opinion. *Klebsiella pneumoniae* certainly is associated with the gastrointestinal tract. As to whether or not *Klebsiella* comes as a normal habitat of the stream, or the soil, is really not relevant. The data are not strong enough to suggest that *Klebsiella* would be there in quantity as it would be from a sanitary source. That sums up what I have found on *Klebsiella*.

Herman: According to some medical and veterinary texts, there is some question as to whether many of these organisms would be classified as *Klebsiella pneumoniae*.

Vasconcelos: At present there are nine binomials (genus and species names) and the rest are referred to as *Klebsiella* serotypes up to 72.

Herman: Out of the 72 serotypes, we now have 68, in the culture bank at Duluth, that come from environmental sources. I am convinced that we shall find them everywhere, depending upon concentrations present.

Knittel: In reviewing the literature, what is your opinion of the validity of the mouse test in proving or disproving pathogenicity? This is one point that has been rather salient with the industry people.

Guarraia: I think we have to look at the reason for using this test. They have used it for *Diplococcus pneumonia*; if it will kill them it is a pathogen. This is then related to encapsulation. I guess in lieu of another test, it is all right. It is very dangerous to extrapolate from a mouse to any other animal because they are exquisitely sensitive to this type of organism.

Bauer: I think from talking to people who do it that we are on particularly shakey ground with mouse pathogenicity test.

Geldreich: As a further insight into this problem, a closely related study was carried out in Argentina in 1956 (Revista de Obras Sanitarios de la Nacion, Vol. 20, p 169-172). It was on bacteria of the *Klebsiella* genus in water, particularly of the La Plata River, which empties near Buenos Aires. They found 61 serotypes of *Klebsiella*. The author cites 14 serotypes from urinary tract infections, 17 from sputum, 2 from fecal material, 9 from abscesses, and 7 from blood infections. He also found them in the La Plata River. This organism is found in feces. It is potentially pathogenic from some people, not everyone, but we certainly have to be concerned about it.

We are concerned about some of these organisms such as *Klebsiella*, *Pseudomonas*, and *Flavobacterium* in drinking water; all three are secondary invaders. They do cause problems in hospitals. We have had a lot of reports around the country that the potable water in hospitals from the organisms get in the whirl baths, the aspirators, the respiratory materials, and patients' wounds. Some very nasty infections result.

Our concern is that as we get higher and higher on the standard plate count, our ability to detect coliforms starts to tail off. It relates to competition from other organisms. I have asked two of our laboratories, in addition to our own, to look at this problem not only in problems of potable water but also in those of paper mill wastes. I want you to be aware that we have some limitations. If you get enough *Klebsiella* in the water, your chances of finding total and fecal coliforms may be suppressed, may be inhibited, or may be reduced significantly so that some of these counts that we get, for instance in paper mill wastes of fecal coliforms, may only represent part of the total number present. I do not want to discuss drinking water here, but this will give you an idea of how we got interested in the problem. We have found in experiments and some few papers in the literature that *Pseudomonas aeruginosa* in certain densities, between 1,000 and 10,000, and upward, actually does suppress the detection of total coliforms in water. *Klebsiella* is apparently another organism that can cause this suppression.

Guarraia: I should like to add one more thing about *Klebsiella*. The reason that these organisms have had a decided growth advantage in pulp and paper wastes is because *Klebsiella* has a very extensive complement of extracellular enzymes. They are able to break down complex carbohydrates that are found in these types of wastes. This is why *Klebsiella* will overgrow so rapidly and other organisms probably can not compete. Also, the data are not available to make the correlation on how many *Klebsiella* you need to cause infection.

Geldreich: Mr. Vasconcelos do you want to pick up the discussion on suppression of indicator organism and describe the work at the Northwest Laboratory?

The Detection and Significance of *Klebsiella* in Water

C. J. Vasconcelos

We got interested in *Klebsiella* from the standpoint of potable waters. Our next step was to go into the influence of the waste liquors on this balance between *Klebsiella* and *E. coli*. I tried first to expose a wild strain of *E. coli* isolated from a river source to a *Klebsiella* strain. I had to have a label.

I needed a rapid way of differentiating *E. coli* from *Klebsiella*. I ran across esculin hydrolysis that is unique for members of the *Klebsiella-Enterobacter-Serratia* group. They apparently possess the ability that *E. coli* does not, to hydrolyze esculin, producing dehydroxy-coumarin which reacts with ferric ions to form a black compound. With this medium I could differentiate *Klebsiella* from *E. coli* rapidly. Also, *E. coli* is motile and *Klebsiella* is nonmotile. I had pure cultures of *E. coli*, a wild strain which I had isolated, and *Klebsiella* Type 22, that was isolated from the stream off the membrane filter. It would have been counted as *E. coli* or fecal coliform by that method. I lypholized the cells so that, in my experiments, I used the same cultures, and they would not have any variation. Before the experiments, I grew the cells in a carbohydrate medium, washed them, and re-suspended them in phosphate buffer. I then determined the cell concentration photometrically. In three flasks, in a shaker bath maintained at 18°C, I had two controls, one containing a concentration of *E. coli*, another a concentration of

Klebsiella, and then the two of them together in the experimental flasks.

I tried these at three different *Klebsiella* levels, maintaining my *E. coli* level at approximately 100 organisms/ml. I had to have a fairly high concentration of *E. coli* because I was working with quite a differential in counts. I was working with 10^2 *E. coli*, as opposed sometimes to 10^7 *Klebsiella*. I had to have a way of picking them out in the same petri dish. In the first experiment I tried them at comparable levels, 100/ml of *E. coli* and 100/ml of *Klebsiella*. With these levels I obtained good results; I could differentiate *E. coli* from *Klebsiella* without any trouble. The same thing was true on M-Endo. When I used bile esculin there was no question which was *E. coli* and which was *Klebsiella*.

In the second experiment I increased the level of *Klebsiella* to 10^5 as opposed to 10^2/ml of *E. coli*. I could recover *E. coli* fairly well by the M-FC, but on the M-Endo the *E. coli* was completely overrun by the *Klebsiella*. By increasing the concentration of 10^7 *Klebsiella* or 10^2 *E. coli*, I could in no way detect *E. coli* with the M-Endo; however, with the M-FC I could. In fact the levels remained almost equal to the control, *E. coli*.

In the last experiment I went on and compared the MF with the MPN method in recovering *E. coli* in the presence of 10^7 *Klebsiella*. I did a correlation based on a ratio of the number of *E. coli* recovered by the MF method, as opposed to the number of *E. coli* recovered by the MPN method. In this first experiment with the *Klebsiella* Type 22 the MPN method appeared to be a little better in recovering *E. coli*. If unity is one, the ratio with the MF method gave a mean of 0.3; with the

MPN method the mean was 0.9. I did not draw any definite conclusions, because I wanted to include other serotypes. It is a good thing I did, because it did not work out that way. However, the other two experiments showed that the MF method used with the fecal coliform medium was better with the MPN method of recovery of *Klebsiella* Type 38. I intended to get the results for the base difference between the two. I started getting higher counts with EC in the tubes containing *Klebsiella* as opposed to the control with the MPN method. I did not get this result as much on the MF method, using the M-FC medium.

Bauer: The *Klebsiella* you had were EC-negative?

Vasconcelos: There are three types of *Klebsiella:* non-aerogenic at 48 hours with good growth, aerogenic strains in EC, and this particular Type 22 which was not aerogenic after 48 hours. *Klebsiella* Type 2 and Type 38 were aerogenic. I differentiated by motility. I inoculated each tube into motility agar; if the stab was all motile, I counted it as *E. coli*. This is how I arrived at my MPN. Basically, it seems that the MF method, with the use of the fecal coliform medium under these experimental conditions, tends to inhibit the *Klebsiella* more than the MPN or the M-Endo medium. The reason for this is not quite clear. I have not explored it, but I presume that it is because the cells were under stress and at this period perhaps *E. coli* could compete more successfully than *Klebsiella* in this medium.

Mr. Bauer, I noted on the data that you presented yesterday that downstream, the *Klebsiella* tend to die off, or one did not recover as many *Klebsiella* as *E. coli*. Now, this may be a consideration. Under stress maybe the cell does not proliferate. I have not carried out any survival studies, but I plan to include some.

As far as differentiating *E. coli* from *Klebsiella*, I think it can be performed easily if this is what the pulp-mill people are interested in. This bile esculine medium works very well with every strain I have ever worked with. They are all esculin-positive. You can almost differentiate *Enterobacter* from *Klebsiella* by motility: one is motile and the other is not.

Geldreich: What is the length of incubation that you use with the bile esculin agar?

Vasconcelos: You can get esculin hydrolysis within one hour. I incubated these for 18 to 24 hours.

Geldreich: Do you think a medium could be developed for a membrane filter procedure?

Vasconcelos: I was thinking about that. I tried taking the filter containing both *E. coli* and *Klebsiella* and placing it on an agar plate containing bile esculin. Within an hour, there was a blackening of the medium; that indicated *Klebsiella* were predominant. With a pure culture of *Klebsiella* placed on the medium, within four hours the colonies had grown out. Another consideration with esculin is that it absorbs in the UV range. If one eliminates the ferric ion from the medium and puts it under a black light, the black zones showing esculin hydrolysis appear distinctly. There is no question about it; whereas, the rest of the medium is fluorescent. There is an article -- in the January 1971 issue of Applied Microbiology, indicating that 99 to 100 percent of the *E. coli* were found to be esculin-negative, whereas the *Klebsiella-Enterbacter*-

Serratia were all positive. I have not subjected this to a comprehensive examination, but to differentiate *E. coli* from *Klebsiella* shows promise.

Guarraia: Have you tried the urease test for this purpose?

Vasconcelos: I have tried that and found too much variation in the strains I tried. Motility and ornithine decarboxylase are the other two tests that are fairly consistent in addition to this esculin hydrolysis.

Knittel: Where do you obtain your esculin?

Vasconcelos: It is available from Difco; I think it is a nutritional biochemical. I found the prepared bile esculin medium that is used for streptococci to be slightly inhibitory to *Klebsiella*, so I used a modified formula with onepercent lactose.

Geldreich: There are two points of interest in what you have presented. One is that we may have a tool to use in work with *Klebsiella*. It may be very helpful for field investigation work because it is not to complicated and could be used in mass sampling field surveys. Secondly, your are beginning to demonstrate that *Klebsiella* in the excessive numbers that we are worried about is actually suppressing our ability to detect indicator systems which in themselves may represent some hazardous situation, whether it is in stream pollution or potable waters, which are obviously not chlorinated.

We need a better indicator system for these tests. We are running into problems with laboratory results around the country, in stream pollution work, potable waters, and source waters. We are running into problems in the southwestern and southeastern parts of our country where there are excessive backgrounds of organisms on the M-Endo MF that are interferring with coliform detection. Frankly we need a better M-Endo

MF medium or a better indicator system than the Schiff reaction of basic fuchsin and sodium sulfite. The problem is occurring in these areas because they have warm water temperatures all the time. I have also known of this problem in the tropics, Puerto Rico, and in the Virgin Islands. In San Paulo, Brazil, some water supplies and surface waters are producing an excessively high background of organisms. We need a better way to suppress these background organisms in order to let the coliforms grow and be differentiated. They are there, but we are losing them.

The problem is not just with the MF methods. The Florida Health Department, for example, has been getting some wildly erratic MPN data in estuarine areas and in some of their fresh water streams in northern Florida. These results relate to the problem that excessive numbers of some of these organisms are actually suppressing coliform detection. Skips in the MPN data and unusual results occur. The most critical problems are with 10 ml and 1 ml inoculations where more of the original sample is present, and oddball results occur like 2, 5, 3, 1 positive tube combinations. Sometimes, they have actually streaked out some of these unusual combinations of negative tubes, at the highest sample amount used, and found a coliform, but it is difficult to isolate. I wanted you to be aware that this problem is occurring not only in the MPN methods but in the MF method because we need some better media. Actually we need chemically defined media, which are more uniform.

Bauer: I should like to direct the conversation back to this pathogenicity aspect and to suggest a person to contact who has been very helpful to us, Dr. John Matsen, (School of Medicine, University of Minnesota, Minneapolis, Minn.) who is extremely knowledgeable about the clinical aspects of infections due to *Klebsiella* as an etiological agent. Dr. Matsen is very cooperative and helpful. He gave a presentation at the meeting in Corvallis and made a couple of important points with respect to whether or not we should consider *Klebsiella* environmental isolates to be potentially harmful. The laboratory at the University of Minnesota Medical School periodically experiences what amounts to localized epidemics of *Klebsiella*. He documented one case recently in the nursery in which 12 infants acquired infections where *Klebsiella* Type 33 was isolated as the causative agent. The sites of infection were quite varied, but eight of those children died. The investigators performed stool isolations from all of the children in the nursery. Upon entering, none of them had *Klebsiella* and shortly thereafter, approximately 39 percent of all these infants were carriers of the organism. They were also able to isolate the organism off the hands of the attending nurses. So, although the rate of infection is low, *Klebsiella*, can be a very virulant organism.

Dr. Matsen described another interesting investigation. He and his co-worker, routinely screen the stools of a number of incoming patients and they find approximately 20 percent are carriers of the organism. Then they compare the number of people who develop a *Klebsiella* infection while they are in the hospital. The people who are pre-colonized prior to entrance run four times the risk of acquiring a hospital-acquired *Klebsiella* infection as those people who are not colonized. Let me emphasize that if these organisms are allowed to proliferate in the environment, we may see an increased rate of colonization among the public, and persons under conditions of stress in the hospital, e.g., antibiotic chemotherapy, catherization, etc., run an elevated risk of acquiring infection.

Guarraia: Another point he brings out is that when you have an anitbiotic regimen even at home, the *Klebsiella* can supplant the normal bacterial flora, because they are not as susceptable.

Geldreich: May I read into the record here a comment Dr. Matsen wrote on this subject, dated June 16, 1974: "*Klebsiella* is a normal flora bacterium in humans although certainly not in the same numbers or to the same extent found with *E. coli*. In spite of being found in the normal flora of fewer individuals than are *E. coli*, *Klebsiella* is more often a cause of septicemia, pneumonia, and serious post-operative infections. *Klebsiella* is the second most common organism, next to *E. coli*, as a causative organism in urinary tract infections. One of the features of *Klebsiella* which makes it a particularly troublesome organism is its propensity to become resistant to anitbiotics. In this regard it is probably our most troublesome hospital bacterium and indeed we have strains at the present time that are resistent to every antibiotic but gentomycin. This worries me as we know that resistance to this particular antibiotic can also occur. I would not recommend that anyone swim, drink, or bathe in water as heavily contaminated as you described. (This is in reference to a problem of paper mill wastes). That *Klebsiella* can cause a primary pneumonia and the aspect of creating a human population with an increased incidence of

Klebsiella colonization are both worrisome to me." These are the critical points which were made and they back up your comments.

Herman: I am curious why that proposal which was requested by Region X and others and which Dr. Matsen submitted to Project Coordination in Washington was not acted upon.

Geldreich: Dr. Johnstone, with the State of Washington, has been very interested in a grant in this particular area also. He has not been able to get funded either.

Knittel: Dr. Johnstone has submitted a grant proposal to study the pathogenicity of *Klebsiella*, cultural comparison of environmental isolation and disinfection techniques. The disposition of the proposal was not known for a long time; it was not funded. I think this is ridiculous. When we got out into the field and meet capable people like this we are continually in the posture of apologizing for the inadequacies of this agency.

Geldreich: "Credibility gap" is the word.

Knittel: Really, it's embarassing.

Herman: It has been more than a year ago that Dr. Matsen submitted his proposal. It is unfortunate that these people with recognized reputations submit proposals to EPA and they wind up lost. Dr. Matsen wants a straight answer. This makes it very embarrassing.

Vasconcelos: In *Streptococcus* work we use the blood agar as an indication of pathogenicity and for staphylococci we use the coagulase reaction. How about *Klebsiella pneumoniae* on DNase medium?

Knittel: Negative

Vasconcelos: Is it negative? Have you tried all these environmental strains?

Knittel: Yes, this is one of our cultural screens. We ran eleven of our strains through the DNase and we have had all negatives up to this point. We did some pathogenicity work with some of our environmental isolates on a very limited basis and we thought we should process them through this capsule medium. If one increases the capsule, one should get increased pathogenicity. Unfortunately they were all negative. However, in a very close observation of the mice during the time they were post inoculated, one could tell they were not feeling too great.

Bauer: I should like to discuss the memoradum, on standardization of methods, that originated with Sid Verner, Office of Monitoring. Are we as microbiologists satisfied with Standard Methods, or what are the areas of dissatisfaction? I should like to poll the group individually as a means of discussing this request and ask whether or not you respond to the memorandum and if you did, what areas you were dissatisfied with.

Geldreich: I will give you the background. A meeting was called by the Office of Monitoring in Washington, on February 10, 1972, to explore the need to develop guidelines in microbiological methods. Kathleen Shimmin had written, under Mr. DeFalco's signature, a strong request to take a look at the needs that we might have for some microbiological methods book within EPA. (Mr. Ballinger might like to prefer to call it an EPA Standard Methods.) The outcome of this meeting was that it was decided to circulate through the Office of Monitoring a memorandum asking each of the Regional Laboratory Directors, each of the Regional Offices, all of the NERC's and anybody we knew within EPA at the supervisory top level to designate one person who would be their leading microbiologist, and ask him/her to respond. Some questions were: What do you see as weaknesses in Standard Methods, are you satisfied with it, and if there are weaknesses, what are they?

We were hopefully going to get this information. I have not received any as yet. It would all be sent to Sid Verner in the Office of Monitoring and he, in turn, would send it out to me. I would separate the comments covering different areas. I would give it to other scientists in the community who are very closely associated with certain aspects in order to collate this material. We would form some sort of a report and then, from there, we would decide what we are going to do. For example, we decided we would ask Dr. Chang to review and collate any comments on protozoans, Gerald Berg on viruses, Francis Brezenski and Bernard Kenner on fecal streptococci, Donald Spino on *Salmonella*, Rocco Russamanno on *Salmonella*, Victor Cabelli on *Pseudomonas*, Richard R. Bauer on *Klebsiella*, and I see, I am also on coliform. Some of you don't even know you have been put on the committee because I did not have any material to send you yet and I did not want to generate something that has not happened.

The idea was to put this in a collated form and then call this somewhat of a "blue ribbon" committee if you want, to make following decisions: first, is there a need for a microbiological-guidelines manual to be circulated among EPA, and secondly, should we try to incorporate the necessary material where it differs from the potable water needs into Standard Methods?

Standard Methods is supposed to cover the needs both for potable water and stream pollution analyses. In the last edition we began to spell out the differences in some of the paragraphs wherever there were divergencies of needs. I hope we continue this in the 14th edition, where there is a definite need to have some separate procedure included, or to perform a test differently because there are problems with bacterial floras, etc. The problems of stream pollution and marine waters are not characteristic of potable waters. Since I am on Standard Methods committee -- as well as Bob Bordner, in this particular group, we should see whethere there are some weak spots (there are always areas for improvement in Standard Methods), and we should certainly try to eliminate them in the development of the 14th edition. I believe you are also on the bathing water committee, Mr. Brezenski?

So, this in the background material. I personally would like to find out which of you have received this assignment, and briefly, what your comments were.

Guarraia: I never received the memorandum.

Comment from the floor: I think it went to the Regional Administrators.

Another comment: It took mine a full month to get to me. It went to the Regional Administrator.

Herman: All I can say is I never saw it.

Bauer: Mr. Davis, can we go on with you?

Davis: First of all, I received the memorandum. My comments were that we have to stick to the routine total and fecal coliform, etc. We don't have any trouble as far as Standard Methods go. I hardly ever read the book. Sometimes things come up, like sample handling procedures. There is a statement in the introduction of Standard Methods, 13th Edition, which says something to the effect that we are not recommending a time condition, but more specific information is given on page 659 of Standard Methods.

Geldreich: I do not recall seeing that. I think that we are going to have to search through this 13th edition. If any of you find areas with which you are dissatisfied, please send them to us. We shall submit proposed changes to the committee for consideration and review. We are asking for your input. Believe me, you are doing us, as well as yourselves, a favor. If possible, we shall try to make Standard Methods more suitable for your needs, as well as more accurate, and have it give us the kind of data we want.

Davis: We are concerned, basically, with sample-handling procedures and quality control. We want to get as valid results as possible. Other than that we have to stick basically to 'tried and true' methods. We have no problems there.

Brezenski: I received the letter and we responded. We have diverse opinions, among the microbiologists in the region, concerning a need for an additional manual besides Standard Methods. Some people felt that Standard Methods was adequate, for the routine parameters, the way it was. We have another faction that felt EPA should have a manual just for the sake of having a manual. My opinion, with respect to this point, was that we really do not need another manual if we are going to just duplicate Standard Methods and put the EPA name on it. I think this is a waste of money and time.

On the other hand, I feel that we need standardization in some critical areas, and whether we do it in EPA or accomplish this in Standard Methods is something to be worked out. I think we need it. This need affects the following areas: (1) bacteriological sampling of sediments, (2) sediment analysis -- what types of samples, sample volumes, and methods should be used, and (3) the dearth of material in Standard Methods concerning the marine environment. These are main areas where we feel that we need some standardization.

The final point here was that if an EPA manual is written, in addition to standard Methods, it should be a representative of new people coming in who have been absorbed from industry, from food, from beverages, etc., and not only the people who have been set in the order for a number of years. We are saying that we need standardized; we do not care whether it is done through Standard Methods or a separate EPA Manual. If we are going to go to an EPA Manual, we should like to have representation from a greater number of people. If we are going to stay with Standard Methods, then I feel Standard Methods is inadequate, as it is, for sediment sampling from the bacteriological point of view and also for the marine environment.

Shimmin: I responded to the effect that I felt that Standard Methods was inadequate as it stands because (1) it certainly lacks in methodology, which I shall go into, and (2) it has to appeal to a wider range of laboratories than we in EPA should have to address ourselves to. For example, it has to include considerations from states that receive their samples in the mail. So, I think Standard Methods needs to be a little less precise than perhaps EPA procedures should be. As far as the methodology goes, I agree with Mr. Brezeuski in the marine area of sediments; there is nothing in there for air; there is certainly a lack in pathogen methodology, e.g., *Vibrio, Shigella,* and *Leptospira*; protozoan methodology is not addressed. In the Southwest (particularly Indian reservations) we have need for such special procedures. Also in Micronesia and in American Samoa, within Region IX, we have great problems with amoebic dysentary. Standard Methods does not concern itself with techniques like fluorescent antibody tests. We need some type of round-robin procedures, something on the order of those used for testing chemical methods. Analytical quality control, including sample handling, is not adequately addressed to in Standard Methods either. I do not think Standard Methods should be discarded; I think it should be separate reference.

Christensen: I have not received the memorandum.

Carroll: We received it two weeks after it was due. We fully intend to respond to it. I think all the points that have already been covered, mainly the one of sample handling time, were an issue among the people in our region. Also they would like to see an expansion of the area of pathogen identification and a section on the fluorescent antibody application. We felt no real critical need for a new manual if it simply would be a duplication of Standard Methods. There are some feeling that Standard Methods was not updated frequently enough and could be supplemented with round-robin tests, as suggested a while ago.

Manhart: Primarily, I have gotten the impression that we should just as well leave Standard Methods pretty much as it is because procedures are covered in a broad spectrum. There were a few places, for example, in the time element for sampling handling where we would stipulate a firmer statement. If a sample is too old, say so, and throw it out. We should leave it like it is, because if we start putting all this information in we have another bacteriological journal. We feel you can go to other texts or literature sources for such information.

Reri: I responded. I feel that Standard Methods is adequate for routine use. It is accepted as having precedence for enforcement. No manual that can be put out would totally meet our needs as far as enforcement goes because we have to play it off the cuff and many times we have to improvise. No methods that could be published could ever fulfill our needs totally.

There is a real need for analytical quality control in EPA. We should have a group that surveys laboratories, as Mr. Geldreich does the state laboratories. The purpose is to help our people in our own organization to make sure that we all comply with Standard Methods especially in the enforcement area.

Winders: Our region received the request and we did reply to the memorandum. I think we agree that the credulance supplied by Standard Methods is something we would not like to see changed by another EPA manual. In our opinion, however, it does not address itself to the areas we work in. We do not work with water supplies. We work with industrial effluents. Invariably, we are trying to locate coliform and fecal coliform bacteria in industrial effluents of very complex mixtures and compounds. Neither the literature nor Standard Methods address themselves in any way to the interpretation of the data we obtained. We realize that there is an influence of these compounds on our data, but we do not know how to interpret the data. We need some help in this respect. I am not sure that there is an answer.

Also, we are very much opposed to the lengthening of the time of sample storage. We would prefer to see testing performed in the field or at small local laboratories. If we do this, of course, we need some sort of quality control techniques. I think our biggest point is that we felt that Standard Methods did not address itself to the types of waters we were working with.

Geldreich: I agree with you we have to work on this area and expand it more in wastewater, which is the second part of Standard Methods. You have got a point.

Brezenski: For example, when you get samples from a meat-packing house they are loaded with grease, fat globules, etc. You treat these samples differently from a normal water sample.

Stang: I did respond in four or five categories, but not in much detail. One specific problem was that there is no recognized standard procedure for sea water (FDA does not even recognize the MF procedure), while there is a standard for the MPN. Another point was that the MF procedure for chlorinated wastewater is specifically denied in Standard Methods. I think everybody uses this. I agree that we do need a more detailed procedure for pathogens, specifically for the people in EPA. My other point was the industrial wastewater guidelines -- we are working on these now.

Bordner: We feel quite strongly about the need for some type of a manual, but I would like to make it clear that we at the Analytical Quality Control Laboratory are not talking about another Standard Methods and I would like to dispel this idea. Any manual of methods and quality control should recognize and closely reference Standard Methods, but I think we could supplement it and really help ourselves by going beyond what Standard Methods offers. The primary objective would be to create a greater uniformity of methodology within our agency. For example, there are certain specific instances where we would like to direct microbiologists to the MF procedure as opposed to MPN; Standard Methods offers both alternatives but does not show this direction or guidance. We would consider this type of manual as a guideline for bench methodology rather than a repetition of Standard Methods.

For pathogens, I think that it might be possible to indicate common procedures that we in the agency could follow but not to the extent that we would restrict ourselves from recognizing natural variation in samples. Some of us have very good information on which of these media work best in water and other samples. How else are we going to get this information than through some type of written communication and more frequent meeting of microbiologists. Perhaps an interim manual could accomplish much along this line. Another point, which we picked up from field personnel, is that microbiologists who are in rather remote areas feel isolated and need a laboratory bench type of commentary that would go beyond Standard Methods and indicate common practice in testing routine and special types of samples. Treatment of industrial effluent samples is a good example of this type of problem.

It seems to me that the reason we really came here today, to discuss the special problems with fecal coliforms and *Klebsiella* in industrial wastes, is a good example of a subject that requires such guidelines. There will be a need for uniformity in methods, for the identification and differentiation of *Klebsiella*, that are certainly not going to be in Standard Methods for some time. Quality control is a big area where we could get together and build such control into an additional or supplementary guideline manual for EPA. Lastly, because EPA's authority goes way beyond water, there may be microbiological involvements in solid waste or air; perhaps we should share information with these areas. We are thinking about a broader scope of manual of guidelines -- call it what you will, that would complement and not replace Standard Methods.

Can the microbiologists who have not had a chance to reply to the memorandum still do so? Do they have to have an official invitation or can they just go ahead and address a reply to Mr. Verner?

Geldreich: I was in hope that they would volunteer. The bench people are working with these problems and they are certainly having experiences about which we would like to hear.

Brezenski: That is the point I make before. We should have a greater representation.

Resi: We have a basic meed for a list of microbiologists; I am talking about all of EPA -- I do not care who they are or where they are. I think that this is one of the ways we can have better communication.

Winders: For instance, we have some microbiologists at Research Triangle, but there is not one of them here.

Brezenski: A directory could be circulated listing several people with their areas of special knowledge or experience. I received such a listing of specialists in solid wastes. They assembled a list of names and, if you have problems in solid wastes, or a particular type of oil, organic compound, or some process, for example, you can contact these people, who are very helpful.

Bordner: That is a great idea. That information could be included in a manual and updated from time to time to help them people who need such contacts.

Bauer: I received the letter and have not responded to it. I will. In general, I agree that if the manual would do nothing other than to reproduce Standard Methods, it would be a waste of time. I must confess that, up until now, I have not given it too much thought, but in coming up last in this discussion and listening to the other points that have been brought up, I shall have to agree with what appears to be a need for a supplemental manual that addresses itself specifically to the problems that are peculiar to this agency, our responsibilities, and our abilities to meet those responsibilities. I would favor something on that order as a supplement to Standard Methods. We certainly need a vehicle in order to get some of this new technology into the hands of the people who need to use it.

Mr. Geldreich, would you like to respond to the questions that have been raised?

Geldreich: There are things that appeared in the 13th Edition which I did not like at all. What bothered me the most were the comments on the MF being used on chlorinated effluents. We wanted to spell out a limitation on using the membrane filter in chlorinated primary effluents. You just cannot use the MF on primary chlorinated effluents.

The membrane filter method works very well on chlorinated secondary effluents or other combinations of treatment, but it is not worded this way in Standard Methods. I have had a series of phone calls from state Health Departments and from private industries selling sewage treatment package plants to the Navy, the Air Force, and the Army and to various cities. All had the same comment: They did not buy this idea that the MF does not work on secondary effluents; I do not either. The method works, and I am sure all of you who have worked with the MF in this better quality of wastewater will agree.

The problem can be demonstrated another way. If one blends the primary effluents sample one will find that one can actually get an increase in coliform count. Part of the reason we are getting erratic results is a gelantinous mass of material plugging the pores. When one blends secondary effluents one does not have these micropellets; one does not have these erratic counts. The MF gives one reproducible results because the secondary is a better quality effluent. We are hoping that we can really show where the limitations on the MF are and make it clear that the MF is applicable to secondary effluents.

The section on swimming waters has a series of *Pseudomonas* tests in it that absolutely do not work. I repreatedly wrote to Archibald Greenburg, even while Standard Methods was in manuscript, and told him about the data collected by our Laboratory and others revealing that the methodology of *Pseudomonas* in the existing Standard Methods is inadequate. (I was not on that subcommittee.)

The Standard Methods committee is divided into several subgroups. Some of the members are from the academic world, some from Federal groups, some from state groups, and some are from city and county health departments. Microbiologists are divided into areas where they have particular experience. For example, Mr. Brezenski may be working on the swimming waters and marine waters and I may be working in another section -- on standard plate count that has to be expanded, or perhaps in this MF section. Mr. Bordner may be working on another part, too. We have specific responsibilities, but we do not have the final decision all the way around on everything that goes into that microbiology section. We are going to try hard to incorporate much of these comments that you are giving us today and those of others who, we hope in the future, will reply to a second invitation.

There are areas on pathogens that need more work. The 13th Edition was the first that had anything on pathogens in that book, and we are hoping to expand that area, certainly for enteropathogenic *E. coli*. We need something on *Leptospira* because we have problems in feed lots. As we have all been saying, we have a need for more expanded methods in wastewater treatment.

A meeting was held April 7, in our office in Cincinnati, with people from the Office of Research and Monitoring. The group included Tom Stanley, Gordon Roebeck, Norm Clark, Earl McFarren, and Robert Clark and took up discussion of the water quality control program. There is a need for a laboratory evaluation program of the EPA laboratories that are involved with field investigations. Many of the data collections that you are making are going to be involved in court actions, in hearings, and in determinations that will affect particular pollution problems. We want to be sure that the data are reliable and that the quality of the laboratory work is beyond reproach. We do not want anybody casting any comments that EPA laboratories are turning out inferior data. We have to protect and update ourselves so that we are all using what we think are the best procedures in order to obtain the most accurate and the most reliable data.

The Analytical Quality Control Laboratory, where you are working, Mr. Bordner, should become involved with the evaluations of the media. For example, we have problems with the commercial medias that are becoming very noticeable around the country. The commercial manufacturers do not turn out a uniform quality media at all times. All of you have seen this problem from time to time. Years ago we tried to set up some kind of a certification program on medium, but we could not do this because, in the Federal Government, it would in a sense be endorsing a product. However, NCCD has found some mechanism whereby they have some control on the serological materials that some of us are using. The center has been able to at least have some quality control worked out. I think this is something that should be included in your program if you have enough man power to do these things.

Bordner: Yes, how do you get a standard madium by which you can test other media? Does any one have any suggestions?

Geldreich: This is one of the problems that we are going to have to solve. In the final analysis if we cannot handle them this way, we are going to have to come up with chemically defined media that will meet our needs in coliform and other tests. For example, we use bile salts; they are variable. Difco makes them one way; BBL makes them another; Oxoid in England makes them a different way. Peptones involve trade secrets. One company makes a peptone one way and goes to a certain point, stops, and calls it Peptone X, while another company boils a little longer, adds a little more of something, and calls it Peptone Y. They are not quite the same, but they use them in our media. As a result, the quality of some of the medias we have developed fluctuates, depending upon which product was used.

We certainly need evaluation of the procedures and equipment. There is going to be more and more instrumentation forthcoming. We need background information which could very well be developed out at your shop, Mr. Bordner, that would help all of us. If we are going to buy some of this equipment, we should like to know what are the desirable specifications; you have been doing it on membrane filters for us since I got out of that area some years ago. You have performed analysis on the waterbath recently and supplied this information. This could be expanded.

Brezenski: You brought up the question about what is a standard medium. Is there a possibility of setting up specifications based on what we think the specifications should be? For example, total coliform media should have a recovery of a given percent using certain organisms. Suppose we have a list of specifications that, we say, have to be met by a medium. If the medium meets these specifications, this is the quality control provided. For example, NCDC has a problem with dye concentrations used in the fluorescent antibody test for beta hemolytic strep. This is creating a big problem in the different diagnostic laboratories. NCDC issued a publication when they evaluated a number of different dyes. They found out that some of the dyes have 10 percent dye and 90 percent inert material -- in others, 30 percent dye. When you make up a 20 percent solution of this dye, it really may not be the same as Allied or some other product. The Center is specifying that the dye must meet this percent concentration, and then, the quality control program is more effective.

Geldreich: This is one of the troubles we have in getting a decent sheen on M-Endo medium. The dye content of the many different lots of basic fuchsin range anywhere from 80 to 99 percent. The inert material is a great unknown; it could be toxic. The concentration of the active ingredients varies. Since we are doing a sulfite-basic fuchsin titration to arrive at the proper proportion to put in the medium for sheen development, it is very important to know the concentration. I do not believe that the manufacturers, once they establish the proportion, continue the quality control. They cut corners because of costs. The quality of these materials fluctuates. We need some way to correct this situation.

Bordner: We have written to BBL and Difco on two occasions about their quality control procedures. BBL provided a detailed description of the Company's quality control. It stated that the firm uses a series of organisms in order to test the water quality media. Difco carries out quality control procedures on all lots of their products, but the

Company did not send prepared descriptions of them. We are suggesting, here, that if we determine the cultures that should be used, it would be a more directly controlled program for which we have some input.

Geldreich: We need to develop specifications or guidelines that we could circulate among EPA laboratories for review and comment.

Resi: They still certify dyes for staining, but certification for our needs is rather fuzzy.

Shimmin: Something else that we need to do is to establish some stronger contacts with Difco and BBL than we have at present. We discovered that the manufacturers slightly changed the formulation of brilliant green agar and did not bother to inform anyone.

Herman: Dr. Gordon with the EPA Laboratory, College Station, Alaska and I found a marked difference among lots of Difco and BBL M-FC media. We wrote both BBL and Difco. We got quick, straight-forward answers back from BBL, but none from Difco.

Resi: We find that we do not achieve the same responses from the organisms, owing to differences in the water characteristics in different geographical areas. There are differences within the medium, but there are also differences in the samples that we are testing.

Herman: I am talking about the same sample in our area versus the medium; not the medium versus various samples.

Brezenski: I feel that we are not really talking about any water that is different in a major way. For example, I would say that when we are testing different types of water, be it fresh or salt water, we have to look at the flora. This is probably the biggest variable that is going to affect our testing for indicator or background organisms and it is going to continue to vary.

We have been receiving reports that a lot of organisms grow, on the M-FC medium, that are not fecal coliforms, when one checks the blue colonies. This is particularly true in warm marine waters and in shellfish areas. We do not see this in temperate waters, for example, off the New Jersey coast in the Atlantic Ocean, where we investigated this problem with the shellfish people. We did some work with the people at the Needham Laboratory to confirm pink and blue colonies on M-FC medium. We obtained a high recovery of fecal coliforms in terms of the blue colony characteristics. The shellfish people say that they get a high percentage of pink colonies that turn out to be fecal coliform and yet, a high percentage of the blue colonies are not fecal coliforms. I think the problem here is the microflora, the indigenous flora in one area.

Geldreich: Part of this relates to the rosalic acid. If the rosalic acid solution is made up and put into that medium within a few minutes, that indicator does cause some color additions to the colonies. It is not an indicator system in a sense, but it produces some non-fecal coliform colonies that yield other colors, such as yellow and red. Rosalic acid should be evaluated.

Periodically the manufacturers come up with a new gadget. One of these gadgets is called the Coli Counter. Of course, I immediately was flooded with requests from our regional offices: "What do you think of this product?" Pan American Health called me and commented: "Say, this looks great. We can send it down with our engineers and they can monitor the (potable) water." The Coli Counter only takes one milliliter, and this is not enough volume to look at potable water; so they recognize immeidately that they cannot use it for that purpose. It does have some crude application as a quick-and-dirty test to determine the density in a stream. Do not use this method when you are trying to establish reliable data. It would give you a rough answer on some surface waters.

I think that you have evaluated these, Mr. Bordner, and I have too. Your data and mine indicate that the Counter has considerable limitations. The service you render in taking a look at some of these products would be of help to the rest of us for use as background material in order to answer questions on this type of subject.

Bordner: The brochures are very appealing, and a lot of people have asked about it.

Geldreich: There are some comments made in the brochures that imply that it is a standard method.

Bordner: They say it is a "standard material," but the implication to some readers is that it is a standard method; it is not!

Winders: We use the Coli Counters when the engineers go into the plants for pre-plant surveys before we do industrial waste surveys. The results tell which outfall stream has sanitary wastes in it. We do not use them as a quantitative method but only to show the presence of that type of bacteria.

Geldreich: If one had to use those results as support data in some court hearing, one certainly is going to have a problem.

Winders: We do not record it as data at all. When we go back in, we sample these streams to run a quantitative analysis.

Bordner: So, we are saying, "Use it, but do not use the data."

Winders: Well, let us say one plant has 15 outfalls; it is better to use this method than to swamp our laboratory with 15 different microbiological analysis samples every day. We find that coliforms are in only two outfalls; then, we concentrate on those.

Bordner: I think that is the type of rough testing that was originally proposed for the Coli Counters.

Geldreich: As a final comment, some of you may have seen a manual called Evaluation of Water Laboratories, which we produced for the evaluation of state and other laboratories that test potable waters. The manual has tied in with the Standard Methods book and with what we considered to be the best operating practices. We are now in the process of rewritting this manual to include not only the bacteriological examination but also the chemical examinations of potable water. Earl McFarren has finished his part. I am expanding the bacteriology to cover not only the needs for potable water testing but also to give the rationale of water quality testing for stream pollution or recreational waters. The manual is about 60 percent finished; it will be about 150 to 200 pages. I hope to finish it by the end of the year. We shall circulate it and we hope that this manual will be of some value, to give you some additional information at the bench level and to explain material that is only vaguely referred to in Standard Methods.

Actually, Standard Methods is a cookbook approach; it does not explain the background for the procedures. We are hoping between this interest in a guidelines' approach that many of us have mentioned here and this new second edition of an Evaluation of Water Laboratories, that we shall be able to give you the supplemental reference material to go along with Standard Methods and that it will be a greater help to you than is Standard Methods alone.

When we do laboratory evaluations, we have a form which we follow, based not only on Standard Methods, but also on our best laboratory practices. We analyze the procedures the laboratories are doing, their equipment needs, the kinds of equipment they have, and the media. We even get into areas involving stream pollution and recreational work now because I have sections in the evaluation form that cover methods for pathogen detection, etc. This type of material is very helpful when we do a laboratory evaluation. The check list is a starting point that we use to make a lot of notes. We then come up with a report covering the capabilities of that laboratory in order to examine not only potable waters but also recreational water. I wanted you to be aware of the manual; it may fit into your concerns about Standard Methods. We hope that we can come out with some material in the future that will be more helpful.

I understand there are 125 EPA facilities scattered around the United States -- not only in the continental area but also in Alaska and other areas of our control. We certainly have to know where these microbiologists are. We would like to identify them, offer our assistance to them, and ask them for ideas from their experiences. I think we are making a start with this meeting and I hope somehow we can find a way to have this kind of meeting once a year. Mr. Bauer, do you have any thoughts on how we can do this?

Bauer: I talked to Mr. Gallagher before the meeting started, and he indicated a certain willingness on the part of the NFIC to sponsor this type of meeting on a regular basis. I certainly think that a meeting of this sort is in order if Field Investigations is willing to assume the responsibility. I intend to talk to Mr. Gallagher about this again if you are in agreement with that approach.

Brezenski: There is only one problem. I attended a meeting on methods in Washington with Mr. Verner's and Mr. Stanley's groups. They may have some ideas along these lines; at least it was made evident at that meeting. I think someone will have to touch base with them. I think we should make it definitely known that if they do not identify people and sponsor such meetings, we will.

Guarraia: I think this purpose is a little different from theirs. You were talking here of addressing problems of microbiologists inclusive...

Brezenski: They would cover the same thing.

Guarraia: Yes, but would all the microbiologists be attending this meeting?

Brezenski: They would make it open to all the microbiologists whom the regions will let go. For example, the region will not let go of every microbiologist they have, but I think it is up to the region or the microbiologist to express his/her desire, to make it important enough so that the administrators are willing to let them go.

Geldreich: We need some sort of a strong recommendation from whoever is going to sponsor the meeting to the people in charge of the microbiologists so that we get the change to go. Some of us may find it rather difficult to convince our supervisors, but if we get the letter from somebody requesting our specific services and explaining the program needs and its value, I think many of us will get the opportunity.

Bordner: This approach from a Washington office would help along that line, would it not?

Geldreich: It worked in this particular instance.

Bordner: Would this invitation be coming from the Office of Monitoring and include microbiologists who are specifically research people or would it include all microbiologists?

Brezenski: It includes both th NERC groups and regional people.

Bordner: If this is going to be a periodic meeting would it be advantageous to meet in different geographical areas of the country, as the large organizations do, to give a better chance for attendance in local areas? Should this be a consideration?

Brezenski: Yes, as they do for chemistry.

Bauer: The permit people do that same thing. They hold meetings in different regions each time. I would like to wrap up the meeting at this time; there are a number of people who have planes to catch and I should like you all to have the opportunity to meet any individuals whom you have not met thus far. I should like to thank you for attending and thank the National Field Investigations Center-Denver for organizing and sponsoring this meeting.

APPENDIX A

CONSIDERATIONS FOR PERMIT PREPARATION PULP AND PAPER INDUSTRY

CONSIDERATIONS FOR PERMIT PREPARATION

PULP AND PAPER INDUSTRY

Effluent Limits

1. Production Basis. The average permitted effluent level, in pounds per day, shall be computed based on maximum daily production, in air-dry tons, as determined at the time of application.

2. Schedule Selection. A permittee with existing abatement program should be at least achieving Attachment B levels and should be put on a schedule to achieve Attachment A levels prior to January 1, 1976. A permittee that is presently without an approved pollution abatement program should be required to expeditously achieve Attachment A levels.

3. Coliform. This is a significant parameter for admixtures of industrial wastewater and sewage. Because of the complex sewering of most mills the absence of sewage must be established by sampling and analysis for fecal coliform organisms. If sewage is present the following effluent limit shall be imposed:

> "Organisms isolated in the fecal coliform test and associated with pathogenes, shall not exceed 1000 organisms per 100 ml (1).
>
> (1) Where receiving waters are classified for shellfish harvesting or contact recreational sports, the effluent limits shall be reduced to comply with the established water quality criteria.

4. Toxic Materials, Oil and Grease. These parameters should be considered to determine their significance on an individual basis. If they are determined to be significant then the appropriate "Special Conditions" should be applied.

5. pH. The pH shall be maintained between 6.0 and 8.5 unless unusual receiving water considerations necessitate a variance (i.e., the natural pH is outside this range).

6. Other Limits. The following may be significant parameters depending on production and receiving water characteristics:

Color
Turbidity

APPENDIX B

TABLES

TABLE I

PULP AND PAPER MILLS STUDIED

Company	Location	Process	Treatment
Publishers	Oregon City, Ore.	groundwood unbleached sulfite magnesium base	primary settling
Boise Cascade	Salem, Ore.	bleached sulfite ammonia base	primary settling
Crown-Zellerbach	Lebanon, Ore.	unbleached sulfite ammonia base	aerated lagoons
American Can	Halsey, Ore.	bleached kraft	aerated lagoons secondary settling
Weyerhaeuser	Springfield, Ore.	unbleached kraft	aerated lagoons

RESULTS

PUBLISHERS-OREGON CITY, ORE.

Source	Date	Verified TC/100 ml	Verified FC/100 ml	FS/100 ml	K. pneumoniae/100 ml
Mill effluent	4/6/70	120,000	3,400	<10	60,000
	4/20/70	70,000	2,200	4	40,000
	5/4/70	82,000	2,000	6	57,000
Willamette River upstream of Publishers Co.	4/6/70	240	4	<10	60
	4/20/70	500	4	<2	<100
	5/4/70	1,400	90	2	600
Willamette River downstream of Publishers Co.	4/6/70	1,200	50	10	500
	4/20/70	200	16	<2	<100
	5/4/70	1,700	380	10	1,000

BOISE CASCADE-SALEM, ORE.

Source	Date	Verified TC/100 ml	Verified FC/100 ml	FS/100 ml	K. pneumoniae/100 ml
Mill effluent	4/6/70	16	<2	<10	<2
	4/20/70	200	2	<2	120
	5/4/70	60	<2	2	20
Willamette River upstream of Boise Cascade	4/6/70	1,000	2	10	500
	4/20/70	2,800	8	<2	1,200
	5/4/70	2,200	90	<2	1,100
Willamette River downstream of Boise Cascade	4/6/70	2,400	1,100	40	200
	4/20/70	1,400	4	2	300
	5/4/70	1,700	90	6	1,000

TABLE I (Cont'd.)

WEYERHAEUSER-SPRINGFIELD, ORE.

Source	Date	Verified TC/100 ml	Verified FC/100 ml	FS/100 ml	K. pneumoniae/100 ml
Mill effluent	4/13/70	4,800	10	<10	2,700
	4/27/70	460	80	<10	400
	5/11/70	2,000	10	10	1,100
McKenzie River upstream of Weyerhaeuser Co.	4/13/70	<2	<2	<10	<2
	4/27/70	210	24	<2	4
	5/11/70	32	14	4	<2
McKenzie River downstream of Weyerhaeuser Co.	4/13/70	12	2	10	8
	4/27/70	37	28	2	20
	5/11/70	45	6	8	10

AMERICAN CAN CO.-HALSEY, ORE.

Source	Date	Verified TC/100 ml	Verified FC/100 ml	FS/100 ml	K. pneumoniae/100 ml
Mill effluent	4/13/70	30	6	<10	6
	4/27/70	33	<10	<2	<10
	5/11/70	1,000	<10	<10	<1,000
Willamette River upstream of American Can Co.	4/13/70	10	2	10	<10
	4/27/70	100	56	2	<20
	5/11/70	210	40	8	<60
Willamette River downstream of American Can Co.	4/13/70	100	10	10	20
	4/27/70	61	54	2	<10
	5/11/70	300	20	8	60

CROWN ZELLERBACH-LEBANON, ORE.

Source	Date	Verified TC/100 ml	Verified FC/100 ml	FS/100 ml	K. pneumoniae/100 ml
Mill effluent	4/13/70	17,000,000	4,200,000	30,000	5,700,000
	4/27/70	26,000,000	90,000	200	14,000,000
	5/11/70	160,000,000	30,000	48,000	130,000,000
S. Santiam River upstream of Crown Zellerbach	4/13/70	44	2	<10	<10
	4/27/70	160	98	87	40
	5/11/70	740	20	28	20
S. Santiam River downstream of Crown Zellerbach	4/13/70	140,000	19,000	140	70,000
	4/27/70	160,000	940	6	100,000
	5/11/70	260,000	140	180	120,000

TABLE II

COLIFORM DATA - EFFLUENT FROM POND I

Date	MPN*/100 mls.	MF**/100 mls.
6/11/69		10,300,000
6/12/69		3,900,000
6/13/69		8,400,000
6/16/69	24,000,000	19,000,000
6/18/69		24,000,000
6/23/69		8,300,000
6/24/69	2,200,000	3,600,000
7/10/69	9,200,000	11,000,000
7/14/69	2,100,000	1,400,000
7/16/69	1,400,000	16,000,000
7/21/69	24,000,000	4,800,000
7/22/69	1,400,000	1,500,000
7/24/69	350,000	400,000

*MPN - Most Probable Number of organisms/100 mls. by the multiple tube method.

**MF - Actual count of organisms/100 ml. as determined by the membrane filter (MF) techniques.

TABLE III

TOTAL COLIFORM
IMViC TYPE DISTRIBUTION IN PULP & PAPER MILL EFFLUENTS

IMViC Types	% @ Publishers	% @ Boise-Cascade	% @ Crown Z.	% @ American Can	% @ Weyerhaeuser	% @ All Mills
----	0	0	*	0	0	*
---+	0	0	*	0	0	*
--+-	0	*	*	0	0	*
-+--	0	0	*	0	0	*
+---	0	0	0	0	0	0
++--	0	*	*	*	0	3.3
-++-	0	*	0	0	0	*
--++	64.3	30.8	48.6	57.1	69.6	52.9
-+-+	*	*	18.9	0	17.4	12.4
+--+	0	0	0	0	0	0
+-+-	0	0	0	0	0	0
+++-	0	0	0	0	0	0
++-+	*	*	*	0	0	5.0
+-++	*	*	0	*	0	4.1
-+++	*	15.4	*	*	*	9.9
++++	*	15.4	0	0	0	5.8

* Insufficient cultures examined.

TABLE IV

FECAL COLIFORM
IMViC TYPE DISTRIBUTION IN PULP AND PAPER MILL EFFLUENTS

IMViC Types	% @ Publishers	% @ Boise-Cascade	% @ Crown Z.	% @ American Can	% @ Weyerhaeuser	% @ All Mills
----	0	*	0	0	0	*
---+	0	*	0	0	0	*
--+-	0	*	*	0	0	*
-+--	0	*	10.8	0	0	4.1
+---	0	*	0	0	0	*
++--	0	*	37.8	0	0	15.3
-++-	0	*	0	0	0	0
--++	89.4	*	37.8	100.0	87.5	68.4
-+-+	0	*	*	0	0	4.1
+--+	0	*	0	0	0	0
+-+-	0	*	0	0	0	0
+++-	0	*	0	0	0	0
++-+	0	*	0	0	0	0
+-++	8.5	*	0	0	0	4.1
-+++	*	*	*	0	*	*
++++	0	*	0	0	0	0

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* Insufficient data.

TABLE V

PERCENTAGE OF AEROGENES IMViC TYPES IDENTIFIED AS KLEBSIELLA pneumoniae IN PULP AND PAPER MILL EFFLUENTS

Source	MF Total Coliform Test % Aerogenes IMViC Types	 % Aerogenes Identified as K. pneumonia	MF Fecal Coliform Test % Aerogenes IMViC Types	 % Aerogenes Identified as K. pneumoniae
Publishers Oregon City, Ore.	64.3	94.4	89.4	90.5
Boise-Cascade Salem, Ore.	30.8	100	*	*
Crown Zellerbach Lebanon, Ore.	48.6	100	37.8	100
American Can Halsey, Ore.	57.1	0	100	75.0
Weyerhaeuser Springfield, Ore.	69.6	68.8	87.5	100
Combined Data	52.9	84.4	68.4	92.5

* Insufficient cultures examined.

TABLE VI

COMPARISON OF TOTAL COLIFORM IMViC TYPE OCCURRENCE FROM VARIOUS SOURCES

IMViC Types	% From Pulp Mill * Effluents	% From Animal a/ Feces	% From Foliage a/	% From Flowers a/	% From Insects a/	% From Undisturbed a/ Soil	% From Polluted a/ Soils
++--	3.3	91.8	13.7	8.4	12.4	5.6	80.6
--++	52.9	2.8	29.6	12.6	10.4	18.8	2.0
-+--		1.5	4.6			3.3	0.2
--+-			1.4	1.7		2.3	
-+-+	12.4	0.6	13.3	14.5	30.6	48.1	13.0
-+++	9.9	0.2	2.4	2.9	2.6	7.7	0.7
++++	5.8	0.1	24.4	24.0	23.4	6.8	
+-++	4.1	0.2	1.4	11.6	4.2	2.9	
++-+	5.0	0.8	4.0	13.7	10.9	3.7	3.3
---+			4.2	9.4	3.9	0.2	0.2
+---		0.1			0.8		
-++-			0.8	0.1			
+--+			0.2	0.6	0.7		
+-+-				0.3		0.3	
+++-		1.9	0.2	0.1		0.3	

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* Combined data from five mills.

a/ Geldreich, E.E., Sanitary Significance of Fecal Coliforms in the Environment., U. S. Department of the Interior - Publication WP-20-3.

TABLE VII

CZ Lebanon, Ore. Fecal Coliform Confirmations

Colony Description	# Picked	% Lactose +	% E.C. +	% Type I E. Coli.
All Blue	71	94	83	60
Blue Centers	16	81	12	9
Light Purple Center	11	100	27	0

TABLE VIII

BACTERIOLOGICAL ANALYSIS OF VARIOUS INDUSTRIAL WASTES AND SURFACE WATERS

DATE	SOURCE	TC/100 ml	No. Colonies Picked	% Lactose	% E.C.+
2-18-71	Jefferson STP Influent	67,000,000	20	75	27
2-18-71	Lebanon STP Influent	10,000,000	20	95	37
2-18-71	Sweethome STP Influent	4,200,000	20	90	67
2-11-71	STP Effluent & Rec.Waters	340	19	50	0
1-20-71	Wapato STP Effluent	1,400	14	79	0
2-18-71	U.S.Plywood Log Pond Overflow	190	19	84	31
2-23-71	Idaho Frozen Foods(Potato)	220,000	19	74	0
7-23-71	ORE-IDA	53,000,000	20	70	27
7-23-71	Simplot	24,000,000	20	45	0
2-23-71	ORE-IDA	16,000,000	20	40	0
7-18-71	IDA Frozen Foods	6,200,000	12	100	42
2-18-71	ORE-IDA	480,000	15	87	15
2-18-71	Simplot	12,000,000	20	95	0
2-18-71	IDA-Frozen Foods	2,000,000	8	88	14
2-10-71	ORE-IDA	13,000,000	20	95	5
2-22-71	Independent Meats	2,000,000	7	57	0
2-22-71	Independent Meats	250,000	20	75	33
1-19-71	H & H Packing Inf.toLagoon	1,400,000	20	55	55
1-19-71	H & H Pacing Effluent	270,000	20	80	63
1-20-71	Northwest Packers	600,000	19	100	0
1-28-71	Kummer Meats	1,100,000	11	100	91

TABLE VIII (cont'd)

DATE	SOURCE	TC/100 ml	No. Colonies Picked	% Lactose	% E.C.+
2-11-71	Fruit Packing Effluent	900,000	14	64	0
2-11-71	Fruit Packing Effluent	6,000,000	20	100	0
2-11-71	Fruit Packing Effluent	2,000,000	20	90	0
2-11-71	Fruit Packing Effluent	1,000,000	14	79	0
1-19-71	U & I Sugar	8,000,000	20	100	5
1-28-71	Alpenrose Dairy	2,500,000	20	100	0
1-28-71	Tillamook Dairy	1,200,000	12	92	67
2-5-71	Ridgefield Wildlife Refuge	400	20	65	23
2-3-71	Ridgefield Wildlife Refuge	400	20	85	6
2-3-71	Ridgefield Wildlife Refuge	400	20	90	40
2-3-71	Ridgefield Wildlife Refuge	400	20	80	18
2-3-71	Ridgefield Wildlife Refuge	1,500	20	10	0
1-28-71	Shadybrook Dump	13,000	13	62	8
1-28-71	Columbia Slough	12,000,000	12	100	100

TABLE IX

BACTERIOLOGICAL ANALYSES OF RECEIVING WATERS

October 1[illegible] Survey

Sta. No.+	Sampling Location	River Mile	Total Coliform Arithmetic Avg/100 ml	Total Coliform Range/100 ml	Fecal Coliform Arithmetic Avg/100 ml	Fecal Coliform Range/100 ml	Fecal Streptococci Arithmetic Avg/100 ml	Fecal Streptococci Range/100 ml
Snake River Above American Falls								
150051	Above Idaho Falls Upper Power Plant	RM 804.7	170	40-300 (4)	13	< 4-20 (3)	20	5-45 (3)
153035	At Grandview Drive, Idaho Falls	RM 801.2	310	190-500 (4)	12	5-20 (3)	92	25-130 (3)
153061	At Broadway Bridge, Idaho Falls	RM 799.9	460	100-800 (4)	13	< 10-20 (3)	220	40-500 (3)
153036	At 17th Street Bridge	RM 799.4	550	100-1000 (4)	10	10 (3)	410	340-480 (2)
153060	Below Idaho ~~[illegible]~~ ~~Falls STP~~ Falls S.T.P. ck	RM 795.0	260,000	4,100->1,000,000 (4)	42	< 4-90 (3)	1000	150-3,600 (3)
150050	Above Shelley at Bennet Bridge	RM 792.3	34,000	13,000-58,000 (4)	87	< 10-140 (3)	420	120-1000 (4)
153059	2 Miles West of Shelley	RM 785.5	31,000	16,000-42,000 (4)	98	25-160 (3)	1,700	200-5000 (4)
153058	Near Firth	RM 780.2	83,000	7,000-20,000 (3)	57	30-80 (3)	770	150-2000 (3)
153057	2 Miles North of Blackfoot	RM 764.7	22,000	10,000-38,000 (4)	77	20-120 (3)	5,300	220-19,000(4)
153056	Near Blackfoot	RM 764.0	24,000	16,000-35,000 (4)	180	30-350 (3)	5,800	160-17,000(3)
153037	At Blackfoot	RM 763.8	36,000	21,000-45,000 (4)	60	40-80 (3)	2,800	100-7,000 (4)
153055	5 Miles Above Tilden Bridge	RM 755.6	17,000	5,400-34,000 (4)	83	20-200 (3)	120	60-210 (4)
150047	At Tilden Bridge	RM 751.0	36,000	20,000-60,000 (4)	440	320-590 (3)	1,300	100-4,100 (4)
153053	6 Miles Below Tilden Bridge	RM 745.0	17,000	5,400-34,000 (4)	83	20-200 (3)	120	60-210 (4)

TABLE X

BACTERIOLOGICAL ANALYSES OF WASTE DISCHARGES
October 1971 Survey

		1	2	3	4	5	6	7	8
				Total Coliform		Fecal Coliform		Fecal Streptococci	
ta. No.+	Sampling Location	Average Flow, MGD	Receiving Stream	Arithmetic Avg/100 ml	Range/100 ml	Arithmetic Avg/100 ml	Range/100 ml	Arithmetic Avg/100 ml	Range/100 ml
53066	Golden Valley Packers RM 812.6	0.3*	Snake R.	160,000(2)	110,000-200,000	28,000(1)	One sample	24,000(1)	One sample
53159	Lewisville Produce RM 815.7/10.6	0.01*	Dry Bed Branch	No data	- -	No data	- -	No data	- -
53082	Idaho Potato Foods RM 804.0	0.7	Snake R.	5,300,000(2)	2,600,000->8,000,000	3,900(2)	310-7,400	>1,000,000(1)	One sample
53081	Western Farmers RM 799.6	0.7	Snake R.	1,700,000(3)	600,000-2,600,000	10,000(2)	10-20,000	1,000,000(2)	1,000,000- 1,000,000
53079	Rogers Bros.(001) RM 799.3	1.1	Snake R.	63,000,000(3)	37,000,000-92,000,000	160(2)	20-300	200,000(2)	25,000-370,000
53167	Rogers Bros.(002) RM 799.2/0.1	0.4	Crow Cr.	55,000,000(2)	>31,000,000->80,000,000	<10(1)	One sample	80,000(2)	41,000-120,000
53166	U & I Sugar Co. Outfall RM 799.2	5.2	Snake R.	4,200(2)	3,500-5,000	10(1)	One sample	990(2)	80-1,900
53080	U & I Sugar Co.	3.6	-	16,000(3)	2,200-43,000	100(2)	10-200	600(2)	400-800

+ Storet Station Number

* Estimated flow

TABLE X (cont'd)

October 1971 Survey

		1	2	3	4	5	6	7	8
				Total Coliform		Fecal Coliform		Fecal Streptococci	
No.+	Sampling Location	Average Flow, MGD	Receiving Stream	Arithmetic Avg/100 ml	Range/100 ml	Arithmetic Avg/100 ml	Range/100 ml	Arithmetic Avg/100 ml	Range/100 ml
067	A & P RM 648.4	0.81	Snake R.	15,000,000(2)	5,500,000-25,000,000	10(1)	One sample	350,000(2)	1,000-710,000
065	Amalgamated Sugar RM 646.9/8.7	8.9	Main Drain	320,000(1)	One sample	772	One sample	110,000(1)	One sample
201	Paul STP RM 646.9/7.2	0.09*	Main Drain	260,000(1)	One sample	No data	- -	5,600(1)	One sample
057	Main Drain RM 646.9/1.8	10.7	Snake R.	19,000,000(4)	7,000,000-47,000,000	44,000(1)	One sample	150,000(4)	20,000-430,000

* Estimated flow

TABLE XI
COMPARISON OF TOTAL COLIFORM IMViC TYPE OCCURRENCE FROM VARIOUS SOURCES

IMViC TYPES	% from Sewage Treatment Plants (data from 5 plants)	% from Potato Waste (data from 9 sources)	% from Meat Packing Effluent (data from 4 plants)	% from Fruit Packing Processes (data from 4 studies)
++--	19.2	2.9	35.3	--
--++	19.2	19.1	13.2	1.8
-+--	2.7	4.8	7.4	1.8
--+-	1.4	0.9	19.1	-
-+-+	13.7	21.0	4.4	3.5
-+++	19.2	23.8	5.9	57.9
++++	8.2	0.9	5.9	7.0
+-++	5.5	14.3	4.4	1.8
++-+	1.4	9.5	1.5	-
---+	-	1.9	-	-
+---	-	-	1.5	-
-++-	8.2	-	-	26.3
+--+	1.4	0.9	-	-
+-+-	-	-	-	-
+++-	-	-	1.5	-

APPENDIX C

EXPERIENCES WITH COLIFORM & ENTERIC ORGANISM ISOLATIONS FROM INDUSTRIAL WASTES

EXPERIENCES WITH COLIFORM AND ENTERIC ORGANISM ISOLATIONS FROM
INDUSTRIAL WASTES, Donald L. Herman, Research Microbiologist
National Water Quality Laboratory, Duluth, Minnesota.

For: Environmental Protection Agency Seminar, THE SIGNIFICANCE OF
FECAL COLIFORM IN INDUSTRIAL WASTES, Denver, Colorado, May 4-5,
1972.

A. Introduction and Review of the Coliforms:

Aquatic microbiologists are presently faced with pertinent, direct, and challenging questions regarding the use of "coliforms" in water quality surveillance relating to standards. Messrs. Geldreich, Butterfield, Wattie, Clark, Kabler, Brezenski, Bauer, and many others have faced these questions in the past, resulting in numerous publications. The microbiologist in EPA is now faced with taking a critical review of oneself, one's work, results, and questions if the "coliforms" can indeed survive the enduring test of time. New microbiologists in our agency must aquire a working knowledge of the literature review (past, present and future needs). They must have a fundamental understanding of chemistry, metabolism, engineering, biology (including fish and other aquatic organisms), and a practical understanding of bacterial survivial. All microbiologists should have field experience and openly exhange information with other EPA microbiologists in order to gain self-confidence. All other parts of the EPA team can be of considerable aid to the microbiologist who is not scared to ask questions. He must be able to define "coliforms" and certainly what bacterial types generally make up the group called "coliforms." The days of "just a coliform count" are not enough when one may be faced with a possible court action.

Brecher and Nestle (1970) in their book entitled "Environmental Law Handbook" state that contamination of water by disease-producing organisms is always a distinct possibility. Bacteriologists look for organisms that inhabit the human intestine, since these are the type that breed the most dangerous diseases. Then, these bacteriologists make the statement "Coliform bacteria are harmless organisms that also inhabit the intestine." This is a comment we hear many times from the parties not wanting to face the possibility of having a high coliform count resulting from their discharge. It is for this reason that a quick review of the "Coliform Group" should be made to refresh our minds. Smith (1969), Morgan (1965), Burrows, and Moulder, Lewert and Rippon (1969), and Merchant (1950) all define the coliform group as *Escherichia coli*, *Enterobacter aerogenes* (Aerobacter), *Kelbsiella pneumoniae* (Friedlander's bacillus), *Paracolobactrum*, *Enterobacter freundii*, *Enterobacter cloacae* and Merchant (1950) adds *Proteus*.

A more precise literature review results in the following facts about each of these "harmless organisms."

E. coli - is unabashed as an opportunist when out its natural settling. It is one of the most common causes of pyelonephritis and urinary tract infections and is an important cause of epidemic diarrhea in nurseries for newborn infants. It is in the intestinal tract of practically all vertebrates and some invertebrates (Geldreich, et al, and Snoddy (1971). Causes "White scours" in calf dysentery (Merchant 1950) and infantile diarrhea (Ørskov 1951). Rall (1970) reports *E. coli* in swine; in household pets, as reported by Meyer, et al (1971), Mian (1959) and Mackel, et al (1960). Pfuhl (1902) reported survival in contaminated soil for 101 days and Kokolios et al (1971) reported survival of *E. coli* in soft agar for 41 years.

Enterobacter aerogenes (aerobacter)- Found frequently in the same environment as E. coli and is also found on grain, plants and soil (Smith 1969 and Geldreich,et.al.). Simonds (1915) used the term "foamy organs" when involved in some types of human infections.

Klebsiella pneumoniae (Friedlander's bacillus)- This organism is not new to the water quality field since it was adopted as a fecal indicator organism in water surveillance in England in the late 1800's (Heukelekian, et.al. 1964). It is considered normal flora of the intestinal tract (Kendall 1916, Morgan 1965 and Hentges 1967). Klebsiella pneumoniae has become known as serious infection when involved in pneumonia with mortality rates reported in humans from 12% to as high as 90% in untreated cases resulting in concern for the young and the aged patients (Baehr 1937, Morgan 1965, and Branson 1968). Blazevic, et.al. (1972) ranks Klebsiella as number two in urinary infections (18.8%) with E. coli being number one (33.9%). Matsen (1970) states that Klebsiella comprise 18.7% of all urinary tract bacterial isolates and the disturbing "propensity" to become resistant to antibiotics which is also confirmed by Burrows,et.al. (1969). Montogomerie, et.al. (1970) review Klebsiella in fecal flora of renal-transplant patients and from other environment sources. He cites cases of infection related to milk shakes having Klebsiella present at the level of 10^5 and finding the organism in potato salad, luncheon sausage, brawn, curry, bacon, porridge, cheese and vegetable mixture, egg custard and in milk shakes thirteen times. Montogomerie states "These results differ from other studies of hospital infections in which predominant Klebsiella serotypes were isolated within an institution and which implicated predominant strains of Klebsiella in the environment as a source of these infections". Burrows, et.al. (1969) cites Klebsiella as paralytic disease of moose and metritis of mares; however, Merchant (1950) states the same cases but clarifies that K. genitalium is found in mares, K. paralytica is related to the moose transfered by ticks and also pathogenic for the bull, sheep and chicken.

Proteus- Proteus morganii is thought to cause infectious diarrhea in infants, Proteus mirabilis is ranked second in urinary tract infections by Smith (1969) and third by Blazevic, et.al. (1972).

Paracolobactrum- Smith (1969) includes the Bethesda-Ballerup group, Arizone group, Providence group, and the Hafnia group which may be associated with the intestinal disease area.

This review then would seem to make a different picture than the one of coliforms being referred to as "harmless organisms", however, one should also remind themselves that almost any bacterial agent in the wrong place at the right time could lead to a infection within the human or animal body.

Mr. Bauer asked that I include the organism Pseudomonas aeruginosa in my discussion, therefore, I have also made a short literature review of this organism.

Ringen, et.al. (1952) found Pseudomonas aeruginosa present in 11% of healthy humans and considered the normal habitat of this organism to be the human intestine. He also found aeruginosa in 90% of sewage samples, 2% of stable manure samples, 3% in soil samples and none in natural waters. Reitler (1957) concluded that this species should be taken into account, as well as E. coli in assessing the suitability of water for drinking and also cited finding 10% present in normal health persons. Hoadley (1968) states concern of aeruginosa in recreational use water related to outer ear infections and suggest that numbers in excess to 100/100mls in swimming waters should be in suspicion. Hoadley further indicates no correlation between populations of E. coli and P. aeruginosa in drinking waters. Hunter, et.al. (1947) cites a epidemic of diarrhea in a new-borne nursey caused by P. aeruginosa resulting in 9 deaths related to contaminated milk supply.

B. Experiences with Coliform and Enteric Organism Isolations from Industrial Wastes and Secondary Effluent:

The majority of the information reported in this section was data collected prior to the time I joined EPA, except the data on domestic secondary effluent and part of the data on paper and pulp. Since I become involved in water pollution in 1962 my interest developed around the enteric survival and isolation both from industry and clinical cases. Since I joined the National Water Quality Lab we have also had a chance to obtain results from some of the nations highest quality natural water.

I must admit it was a rude awakening when one realizes how little "mother nature" has paid attention to both the clinical and engineering text books regarding survival of the enterics in natural receiving waters. The engineering courses I had covered the coliforms very quickly and from that point on we discussed the bacterial biological mass related to loading, flows, retention times and general disinfection (usually in % reduction).

The types of industrial waste I became involved with includes the following- Canning and Food Processing, Beverage- carbonated and non-carbonated, potato waste, Meat and Slaughter Waste, Paper and Pulp Waste and finally Domestic Secondary Effluent. I was soón to find out that the controling parities did not want to know what levels of enteric organisms may be present in their waste but wanted strictly just "coliform" counts, if at all. It was not until the states involved or Federal action was indicated that the enteric information was wanted and then they wanted a years study done in 30 days with no written reports unless it was in the favor of the industry concerning their final discharge. I am glad to report that this was not the case concerning all industries I worked with but the majority can be included.

The following tables show the levels of enteric and indicator organisms we found in these various waste. The improvement in isolations from water relates directly to the improved methods resulting from the use of the membrane filter and media developed in the more recent years as understanding of the microbes within the aquatic environment improved. The items that are strongly indicated is the type of nutrient addition, colloidal suspension, [illegible] bottom sludge development and seed organisms.

1. Canning and Food Processing Waste:

a) Initial stage of aerated lagoons

Initial BOD, mg/l - 1,400 (range: 160 - 2,400)

Flow rate - 125,000 gpd

Total coliform/100 ml - 320,000 (range: 2,000 to 1,500,000)

Fecal coliform/100 ml - 15,000 (range: 100 - 60,000)

(1) Results of Fecal Coliform Isolations

	percent
E. coli, type I -	35.0
Klebsiella pneumoniae	55.0
Enterobacter species	3.3
Pectobacterium	6.0
**Salmonella* species	0.7

b) Post-treatment effluent - discharged without chlorination

Final BOD, mg/l - 30 (range: 6.0 - 180), 97.8% reduction

Retention time in aerated lagoons - 21 days

Total coliform/100 ml - 3,000 (range: 800 - 7,000)

Fecal coliform/100 ml - 140 (range: 20 - 500)

(1) Results of Fecal Coliform Isolations

	percent
E. coli, type I	32.1
Klebsiella pneumoniae	42.9
Enterobacter species	5.0
Pectobacterium	9.3
**Salmonella* species	10.7

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* Random isolates were confirmed as *Salmonella typhimurium* and 12 isolates, confirmed of *Shigella sonnei*.

2. Beverage Waste-(carbonated and non-carbonated):

a) Initial stage of aerated lagoons

Initial BOD, mg/l - 1,600 (range: 800 - 18,000)

Flow rate - 36,000 gpd

Total coliform/100 ml - 7,500,000 (range: 15,000 - 64,000,000)

Fecal coliform/100 ml - 50,000 (range: 2,500 - 750,000

(1) Results of Fecal Coliform Isolations

	percent
E. coli, type I	5.6
Klebsiella pneumoniae	68.0
Enterobacter species	15.0
Pectobacterium	7.0
*Salmonella species	4.4

b) Post treatment effluent - discharged without chlorination

Final BOD, mg/l - 75 (range 6.1 - 160), 95.3 % reduction

Retention time in aerated lagoons - 14 days

Total coliform/100 ml - 4,500 (range 15 - 230,000)

Fecal coliform/100 ml - 1,700 (range 5 - 75,000)

(1) Results of Fecal Coliform Isolations

	percent
E. coli, type I	20.6
Klebsiella pneumoniae	67.0
Enterobacter species	4.7
Pectobacterium	5.8
*Salmonella species	1.9

---

* Random isolates were confirmed as Salmonella typhimurium.

3. Potato Waste:

a) Initial stage of aerated lagoons

Initial COD, mg/l - 14,000 (range: 1,500 - 45,000)

Flow rage - 75,000 gpd

Total coliform/100 ml - 160,000,000 (range: 2,500,000 - 750,000,000)

Fecal coliform/100 ml - 265,000 (range: 18,000 - 850,000)

(1) Results of Fecal Coliform Isolations

	percent
E. coli, type I	0.9
Klebsiella pneumoniae	81.1
Emterobacter species	9.4
Pectobacterium	6.9
**Salmonella* species	1.6

b) Post treatment effluent - discharged without chlorination

Final COD, mg/l - 140 (range: 170 - 3,500), 99% reduction

Retention time in aerated lagoons - 41 days

Total coliform/100 ml - 1,700 (range: 220 - 18,000)

Fecal coliform/100 ml - 45 (range: 7 - 11,000)

(1) Results of Fecla Coliform Isolations

	percent
E. coli, type I	15.5
Klebsiella pneumoniae	60.1
Enterobacter species	8.9
**Salmonella* species	15.5

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* Random isolates were confirmed as *Salmonella typhimurium* and *Salmonella St. paul*.

4. **Meat and Slaughter Waste:**

a) Initial stage of aerated lagoons

Initial BOD, mg/l - 26,000 (range: 2,600 - 45,000)

Flow rate - 28,000 gpd

Total coliform/100 ml - 670,000,000 (range: 3.5 mill. - 8.5 bill.)

Fecal coliform/100 ml - 6,500,000 (range: 1.5 mill. - 1.2 bill.)

(1) Results of Fecal Coliform Isolations

	percent
E. coli, type I	56.9
Klebsiella pneumoniae	21.5
Enterobacter species	13.8
Pectobacterium	0.5
**Salmonella* species	7.3

b) Post-treatment effluent - discharged without chlorination

Final BOD, mg/l - 400 (range 150 - 2,200), 98.5% reduction

Retention time in aerated lagoons - 36 days

Total coliform/100 ml - 250,000 (range 17,000 - 1,300,000)

Fecal coliform/100 ml - 18,700 (range 7,800 - 65,000)

(1) Results of Fecal Coliform Isolations

	percent
E. coli, type I	65.8
Klebsiella pneumoniae	24.1
Enterobacter species	6.9
Pectobacterium	0.9
**Salmonella* species	2.3

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* Initial random isolates were confirmed as *Salmonella typhimurium* and *St. paul*. Resampling confirmed these findings and added var. *copenhagen* as well as *Shigella sonnei* and *flexneri*. Orders were then passed that no more *Salmonella* or *Shigella* isolations should be done because of reports to NCCD.

This was a interesting case in that wind draft carried organisms from the aeration lagoons at times into the plant resulting in loss of finished goods and contamination of entire areas of the plant to allow processing to continue.

5. Paper and Pulp Waste:

This phase of work resulted in some real challenges with the utmost use of tact and diplomatic relationship in order to obtain valid samples. It was a question whether any of the samples representing full production stress.

a) Initial stage of aerated lagoon

Flow rate - unknown, Initial BOD, mg/l - 2,200 (range: 35-4,500)

Total Coliform/100 ml - 1,500,000 (range: 500,000 - 5.7 mill.)

Fecal Coliform/100 ml - 8,000 (range: 10 - 87,000)

(1) Results of fecal coliform isolations.

	percent
E. coli, type I	4.4
Klebsiella pneumoniae	85.0
Enterobacter species	9.5
Pectobacterium	0.8
**Salmonella* species	0.3

b) Post-treatment - discharged without chlorination.

Final BOD, mg/l - 3,600 (range: 400 - 6,000), approx. 64% increase

Retention time in aerated lagoon - 2 days (claimed), 4' discharge pipe.

Total Coliform/100 ml - 57,000,000 (range: 2-79 mill.)

Fecal Coliform/100 ml - 130,000 (range: 60 - 750,000)

(1) Results of Fecal Coliform Isolations

	percent
E. coli, type I	0.4
Klebsiella pneumoniae	92.3
Enterobacter species	6.7
Pectobacterium	0.6
**Salmonella* species	0.008

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* Random isolates confirmed as *Salmonella typhimurium*.

Field survey work has been done on one of the rivers used by the industry for discharge of the paper and pulp waste. This resulted in demonstrated fish kills due to lowered DO in 1969 and 1970, COD from 12 to 260, Total Coliform/100 ml ranges from 10 to 171,000 with Fecal Coliform/100ml ranged from 3 to75,000 and Fecal Strep./100 ml ranged from 6 to 1,350. Eleven type of pathogenic bacteria were found and confirmed by CDC including *Klebsiella pneumonia*, *Shigella sonnei*, *Salmonella typhirmurium*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. We found one isolate of *Klebsiella pneumonia* above the major discharge area while recovering types 14, 58, 21, 22, 31, 33, 35, 65, 7, 56 and 67 up to and including 27 river miles below the outfall. Within the river we found active "colonized" zones of *Klebsiella pneumonia* types 14, 58, 21, 22, and 31. These types were tested for mouse pathogenicity and were shown to be positive. In the zones of "colonization" bottom rocks were recovered that had pure cultures of *Klebsiella pneumonia* growing on the surface edges. The chemistry factors of interest in these areas were- decreased DO, none for some 7 miles, decreased pH of the water and presence of reducing and hexose sugars. *Salmonella* was found once when the fecal coliform was 3/100 ml and always when the fecal coliform was above 40/100 ml. The fecal strep. to fecal coliform ratio ranged from 1:14.6 to 325:1. Bottom samples below area of discharge ranged from 50,000 to 5,600,000 in total coliform; fecal coliform ranged from 300 to 23,000, both test related to 100 mls of interface water samples. In all bottom samples *E. coli*, type I, *Klebsiella pneumonia* and *Salmonella typhirmurium* were demonstrated.

6. Secondary unchlorinated effluent:

Total coliforms/100 ml	581,000
Fecal coliforms/100 ml	32,500

(1) Results of Fecal Coliform Isolations

	percent
E. coli, type I	62.0
Klebsiella pneumoniae	18.0
Enterobacter species	14.3
Pectobacterium	3.6
Salmonella species	2.1

This waste has been used in disinfection and fish effects from tanks receiving the various effluents. Presently the data is being processed and reviewed for publication by staff members at a later date.

7. Antibiotic Sensitivity Results on Random Selected *Klebsiella pneumoniae* Isolates are shown on the attached tables.

Pseudomonas aeruginosa - Test were conducted for this organism resulting finding in all cases ranging from 3/100 mls. to 6,700/100 mls. This area became a major work load due to isolation and confirmation with a limited staff. Hektoen Agar (King 1968) was used which many times resulted in a coin toss as to what the colony was really indicating. We have found *Pseudomonas aeruginosa* present in low numbers where fecal coliforms were not detected and also have found the fecal coliforms and Salmonella when *Ps. aeruginosa* was not found. I agree about some concern related to this organism but prefer to think of it as a working part of the total picture and not just the "only" indicator.

ANTIBIOTIC SENSITIVITY RESULTS ON RANDOM SELECTED *Klebsiella pneumoniae* ISOLATES, NWQL, EPA

Isolation Source	Kleb. Type (if known)	Sulfathiazole 0.25 mg	Streptomycin 10 mcg	Gantrisin 2.0 mg	Chlorampenicol 5 mcg	Furacin 100 mcg
# 363-d-3 Sec. Effl.ST	at CDC	S	I	R	S	S
# 363-e-3 Sec. Effl.ST	at CDC	S	S	R	S	S
# 365-b-3 Cl.Sec.Effl.	at CDC	S	R	R	S	S
# 365-h-3 Cl.Sec.Effl.	at CDC	S	R	R	S	S
# 366-k-3 DeCl.Sec.Effl.	at CDC	I	S	R	S	S
# 371-c-3 DeCl.Sec.Effl.	at CDC	I	S	R	S	S
# 426-g-3 Ozone Sec.Effl.	at CDC	I	I	R	S	S
# 426-h-3 Ozone Sec.Effl.	at CDC	I	S	R	S	S
# 429-c-3 Sec. Effl.	at CDC	S	S	R	S	S
# 489-3 Lake Superior Duluth Intake	at CDC	S	I	R	S	S
# 1-6 Paper & Pulp	19	R	R	R	S	S
# 24-7 Paper & Pulp	19	R	R	R	S	S
# 24-12 Paper & Pulp	33-35	R	R	R	S	S
# 31-15 Paper & Pulp	47	R	R	R	S	S
# 31-16 Paper & Pulp	47	R	R	R	S	S

R = Resistant, I = Intermediate and S = Sensitive

Continued- ANTIBIOTIC SENSITIVITY RESULTS ON RANDOM SELECTED *Klebsiella pneumonia* ISOLATES, NWQL, EPA

Isolation Source	Kleb. Type (if known)	Polymyxin B 300 units	Neomycin 30 mcg	Cephalothin 30 mcg	Tectracycline 30 mcg	Ampicillin 10 mcg
# 363-d-3 Sec.Effl.ST	at CDC	I	NT	S	I	R
# 363-e-3 Sec.Effl.ST	at CDC	I	I	S	S	S
# 365-b-3 Cl.Sec.Effl.	at CDC	I	I	LA	I	I
# 365-h-3 Cl.Sec.Effl.	at CDC	I	I	S	S	R
# 366-k-3 DeCl.Sec.Effl.	at CDC	I	S	S	S	R
# 371-c-3 DeCl.Sec.Effl.	at CDC	I	I	S	S	R
# 426-g-3 Ozone Sec.Effl.	at CDC	I	I	S	S	S
# 426-h-3 Ozone Sec.Effl.	at CDC	I	S	S	S	S
# 429-c-3 Sec. Effl.	at CDC	I	S	S	I	R
# 429-x-3 Sec. Effl.	at CDC	S	S	S	S	I
# 489-3 Lake Superior Duluth Intake	at CDC	I	I	S	I	R
# 1-6 Paper & Pulp	19	R	R	S	LA	R
# 24-7 Paper & Pulp	19	R	R	R	R	R
# 24-12 Paper & Pulp	33-35	I	R	S	R	R
# 31-15 Paper & Pulp	47	I	R	S	I	R
# 31-16 Paper & Pulp	47	R	R	S	I	R

R = Resistant, I = Intermediate and S = Sensitive

Summation:

Our experience seems to indicate that the bacterial flora of receiving waters and the behavior of intestial track flora may have some interesting relations. The addition of too much nutrients and substrate can totally disrupt the normal human natural flora balance. Infants and adults when fed cheese or apple cider in excess have resulting problems. Kendall(1916) discussed this nutritional effect. High quality water is also extremely low in total coliforms and void in many areas of fecal coliforms, except below beavor ponds, shore birds, etc. These enterics found usually do not survive long in cold, high quality fresh water without proper substrates.

The adverse effects by nutrient and waste addition are clearly shown by Gallagher, et.al. (1970), Arthur,et.al.(1969), Anderson, et.al.(1971), USDI,FWPCA (1967), US HEW,PHS (1966 & 1965) relating to waste in Puget Sound, Slime Growth in Pulp Waste, Combined Waste, Pollution of Mobile Bay and Pollution on the Red River of the North. One must remember that these are just some of the numberous reports already published.

The coliform areas have been well reviewed and tested since 1904 by Geldreich,et.al. (some 16 major articles in my file), Van Donsel, Gordon (1970), Smith and Twedt (1971), Senn, et.al. (1963) and many others. The text entitled "Sanitary Significance of Fecal Coliforms In The Environment by Geldreich (1966) continues to answer questions each time I review this reference.

We are presently under planned, well organized criticism by some of the major industrial and other large volume effluent sources. The time for EPA microbiologist to become well organized is long overdue. We must work together, exchange information, have a excellent working knowledge of literature reviews and above all obtain facts with a cool head. Review of E. coli isolations showed that 97.2 % of these were E. coli, type I. In all cases when fecal coliform counts were greater than 100 per 100 mls. Salmonella was recovered. Eickhoff (1972) on Klebsiella points out the critical need for epidemiology work on Klebsiella pneumonia in recreational waters. A proposal of this type was submitted by Dr. John Matsen in 1971, #HMA and has been lost in Washington. The need for such is present even greater now.

The developing legal actions across the nation seem to indicate the need for a bacterial identification unit in EPA. We must be able to determine more often what constitutes many of the organisms found in the various coliform counts.

The plain fact remains at present microbiologist must become well organized and work closely with the chemist, biologist, engineers, adminstration and ourselves. I basically believe EPA has the start of a great team therefore we should make full use of any and all facilities and talent at our finger tips.

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APPENDIX D

IDENTIFICATION OF COLIFORM COLONIES

TABLE I

Identification of Total Coliform Colonies
Selected Industrial Wastes

	Concentration		Colony Identification						
	Coliforms*/100 ml				Enterotube				
Source	Total	Fecal	Colony° Description	Total No. Colonies	Klebsiella-Enterobacter liquifaciens	Other Entero-bacteraceae	Non Entero-bacteraceae	EC (+)	BGBL (+)
Sugar Waste Bone char filter Washings	11,000	<67	Metallic Green	24	18	4	2	2	24
Sugar Waste Treatment Plant	8,000	<67	Metallic Green	23	21	0	2	1	23
Pulp Mill Process Water	23,000	>60,000	Metallic Green	24	23	1	0	1	24

* Typical colonies on M-Endo and M-FC Agar

° M Endo Medium

TABLE II

Identification of Various Colonies
From the Fecal Coliform Membrane Filter Test
Selected Industrial Wastes

	Concentration		Colony Identification						
	Coliforms*/100 ml				Enterotube				
Source	Total	Fecal	Colony° Description	Total No. Colonies	Klebsiella-Enterobacter liquifaciens	Other Enterobacteraceae	Non Enterobacteraceae	EC (+)	BGBL (+)
Sugar Waste Filter Washings	11,000	<67	Gray-white	18	15	3	0	1	18
Sugar Waste Treatment Plant	8,000	<67	Gray-white	14	14	0	0	0	14
Sugar Waste Treatment Plant			Blue	7	6	1	0	2	7
Pulp Mill Process Water	23,000	>60,000	Pink	24	7	1	16	4	15
Pulp Mill Process Water			Blue	22	3	3	16	3	19
San Joaquin R. Stagnant Area	<67	>6,000	Gray-blue	24	1	3	20	0	8
Pulp Mill Cooling Water	<67	<67	White	23	0	18	5	0	3

* Typical colonies on M-Endo and M-FC Agar
° M-FC Medium

APPENDIX E

FIGURES

FIGURE I

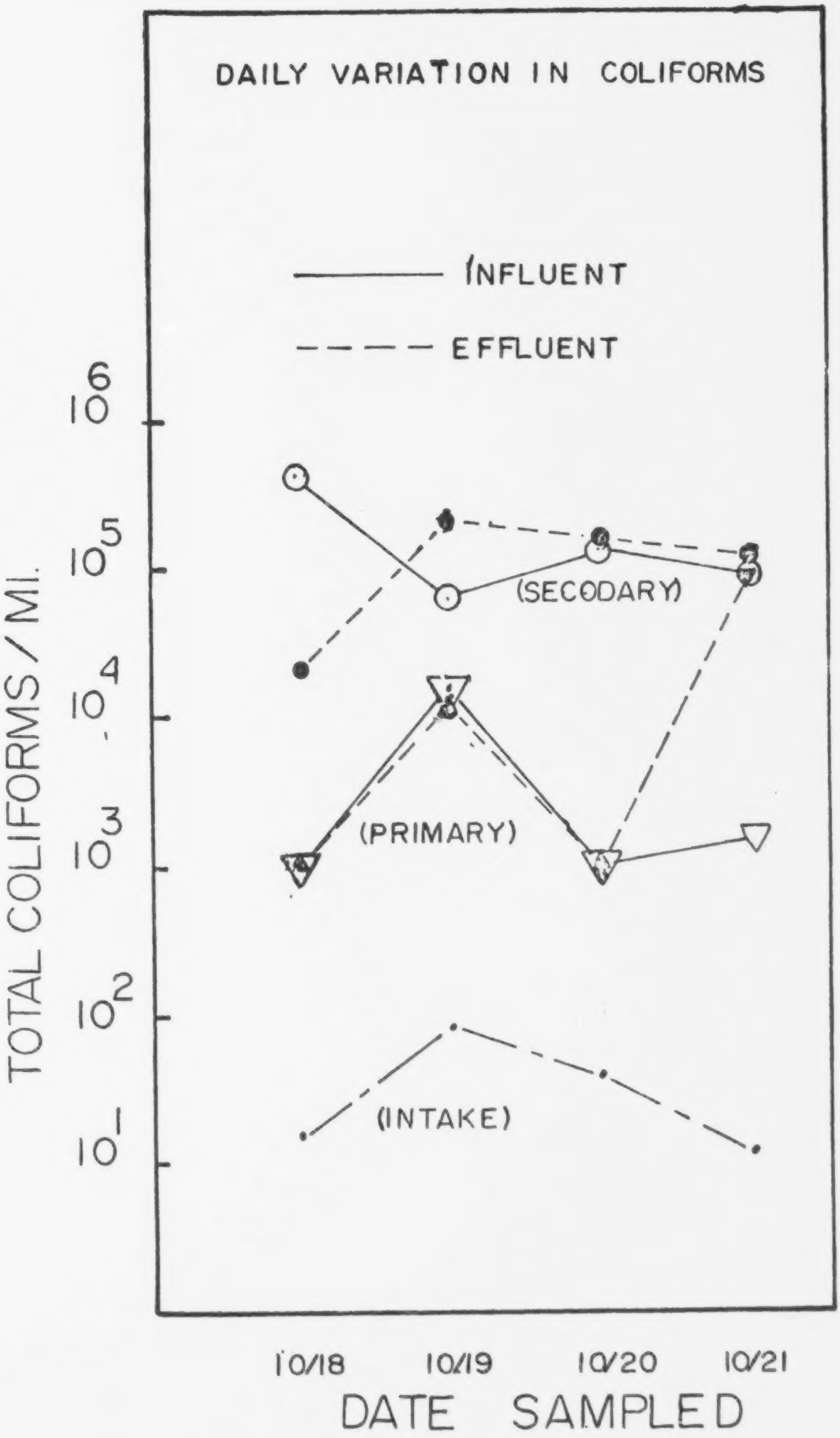
DAILY VARIATION IN COLIFORMS
INFLUENT
EFFLUENT
TOTAL COLIFORMS / MI.
10^6
10^5
10^4
10^3
10^2
10^1
(SECODARY)
(PRIMARY)
(INTAKE)
10/18
10/19
10/20
10/21
DATE SAMPLED

FIGURE 2

Composition of Total Coliform Population Found in Pulpmill Wastes

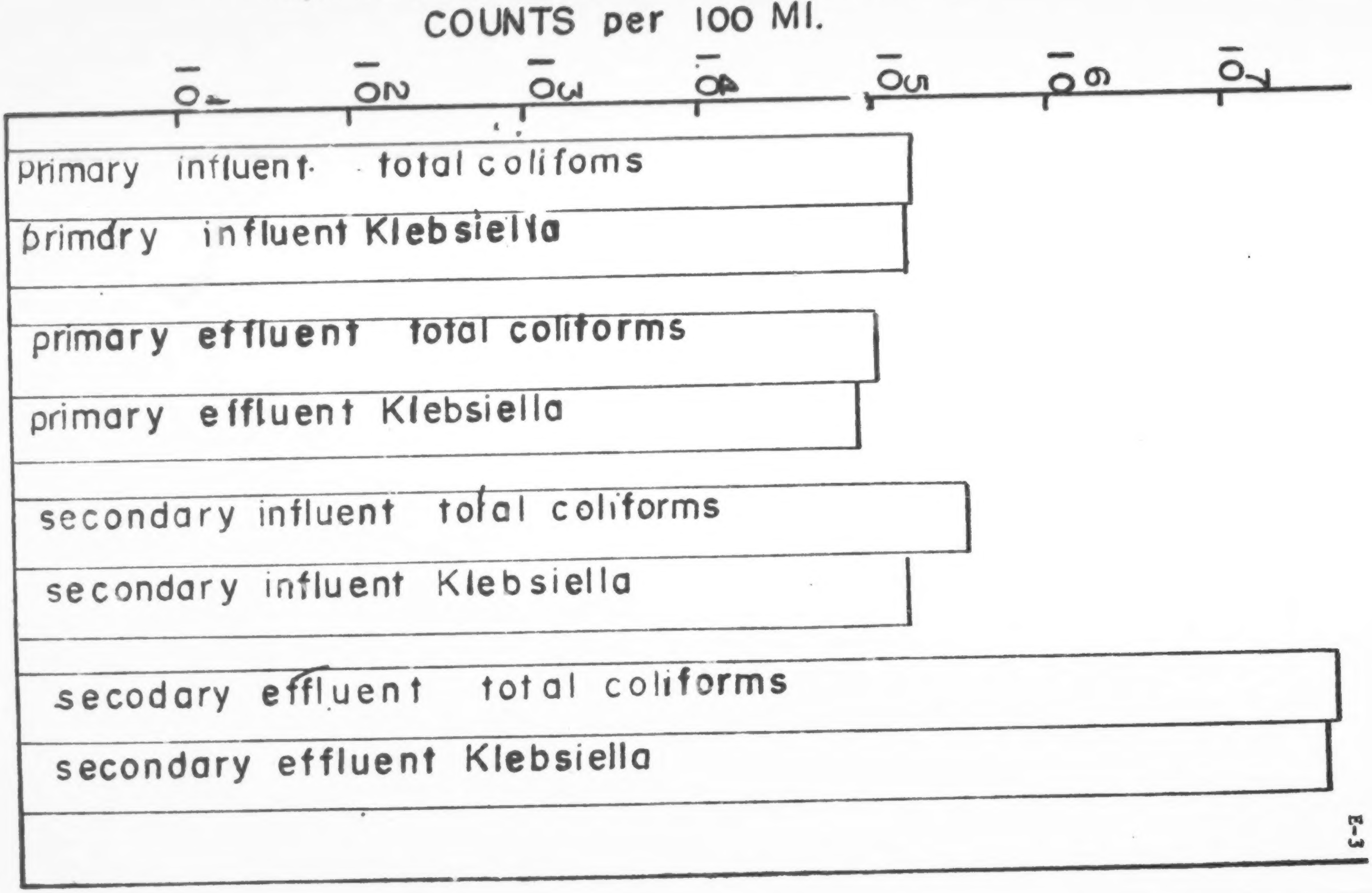

FIGURE 3

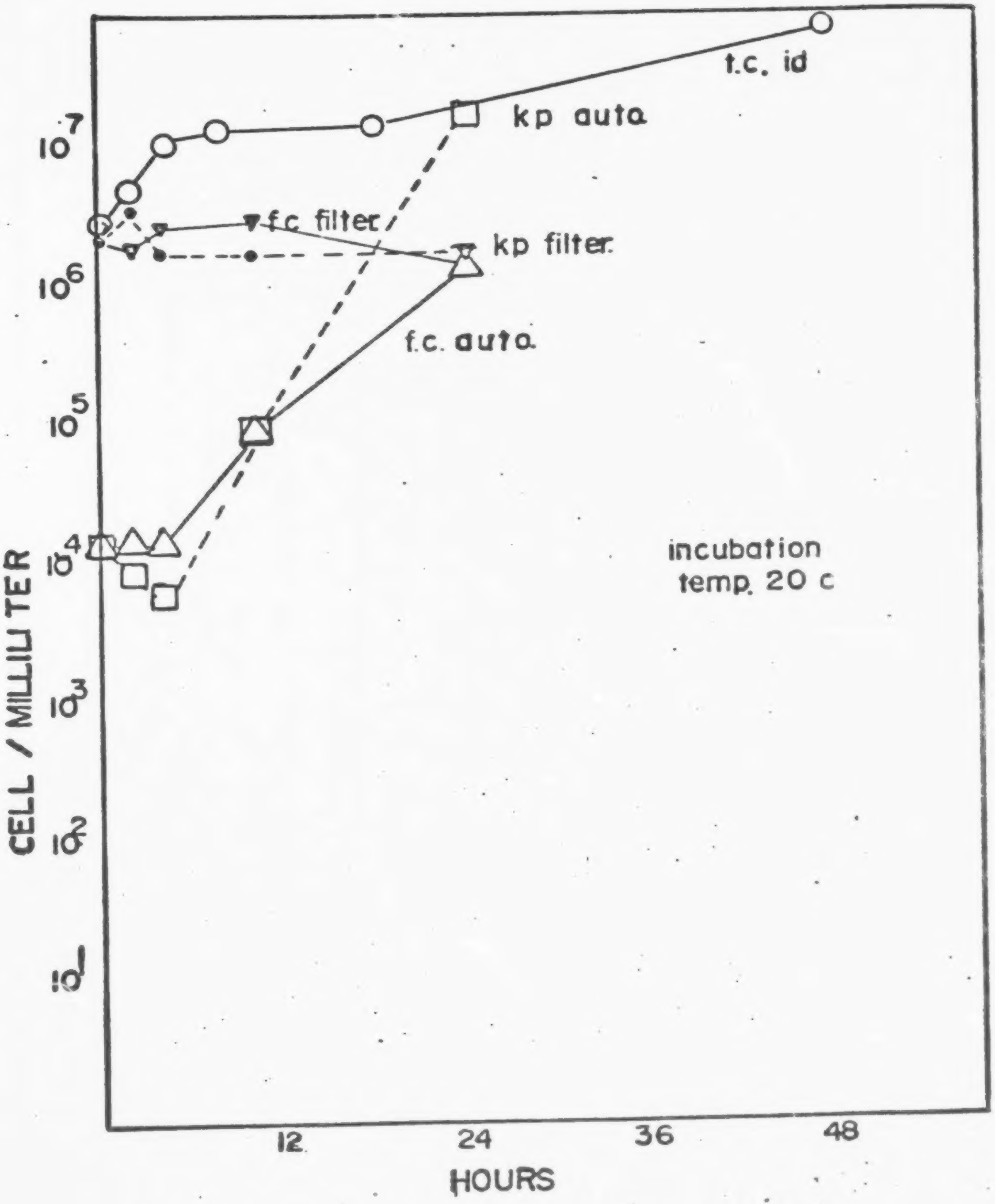
t.c. id
kp auto
f.c filter
kp filter
f.c. auto
incubation temp. 20 c
CELL / MILLILITER
10^7
10^6
10^5
10^4
10^3
10^2
10^1
12
24
36
48
HOURS

FIGURE 4

ENVIRONMENTAL COLIFORM COUNTS /100 Ml.

Sample	Total coliform	Feceal coliform	Feceal strep
lettece	0	0	0
pine need.	0	0	0
leaves	0	0	0
leaves[1]	0	0	0
soil	0	0	0
water	1×10^3[2]	0	0

[1] DECAYED LEAVES

[2] 48 HOURS INCUBATION, NONE FERMENTED LACTOSE AFTER 48 HOURS

Effect of Temperature on Recovery of Fecal Coliforms by the Membrane Filtration Method - FIGURE 5

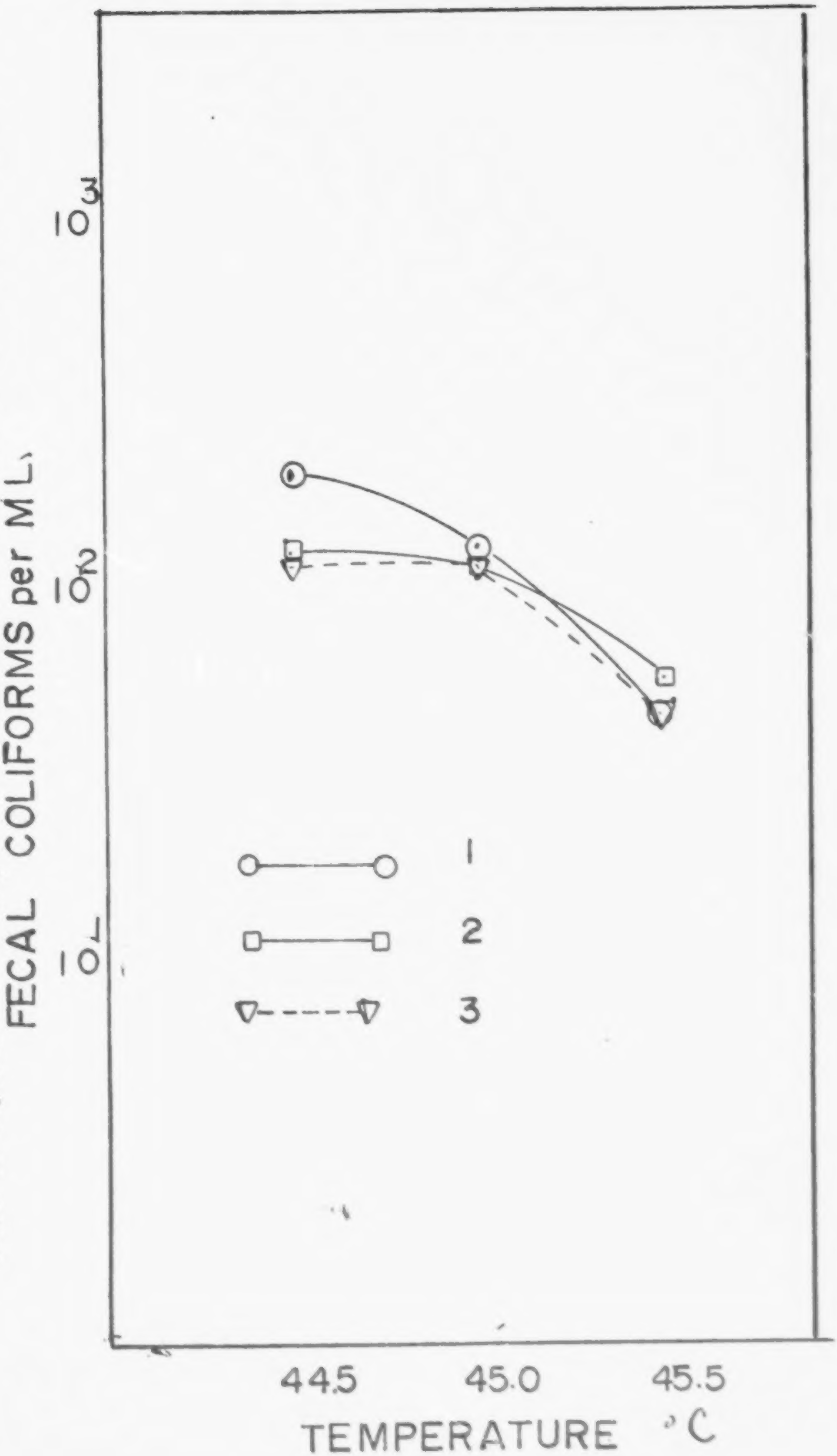

FIGURE 6

	dark blue	light blue	44.5		45.0		45.5	
			BLUE	LIGHT BLUE	BLUE	LIGHT BLUE	BLUE	LIGHT BLUE
INDOLE	100[a]	0	100	0	100	0	100	0
M.R.	100	9	100	25	100	25	100	0
V.P.	0	90	0	100	0	100	0	0
CITRATE	14	100	0	100	0	100	0	0
GLUCOSE	100	96	100	100	100	100	100	0
LACTOSE	100	100 (48 hour)	100	100	100	75	100	0
SUCROSE	—	—	100	100	75	75	100	0
MANNITOL	—	—	100	100	100	75	100	0
ACETATE	—	—	75	25	75	25	75	100
LYSINE	88	-	75	100	100	100	100	100
ARGININE	—	—	25	0	75	50	75	---
ORNITHINE	-	—	75	0	50	50	100	-
UREA	—	—	0	100	0	100	0	75
MOTILITY	—	—	75	0	100	0	100	0
LACTOSE (445 C)	89	0						

[a] numbers are per cent of cultures showing positive reaction